Discovery of psychoactive plant and mushroom alkaloids in ancient fungal cicada pathogens

Greg R. Boyce, Emile Gluck-Thaler, Jason C. Slot, Jason E. Stajich, William J. Davis, Tim Y. James, John R. Cooley, Daniel G. Panaccione, Jørgen Eilenberg, Henrik De Fine Licht, Angie M. Macias, Matthew C. Berger, Kristen L. Wickert, Cameron M. Stauder, Ellie J. Spahr, Matthew D. Maust, Amy M. Metheny, Chris Simon, Gene Kritsky, Kathie T. Hodge, Richard A. Humber, Terry Gullion, Dylan P. G. Short, Teiya Kijimoto, Dan Mozgai, Nidia Arguedas, Matt T. Kasson*

*Corresponding author. Email: mtkasson@mail.wvu.edu (M.T.K.)

This Supplementary Materials PDF file includes:

Supplementary Results

Morphological studies

Morphological studies were conducted to permit species-level identification of collected specimens. Conidia (n = 37) and resting spore (n = 14) measurements were acquired for freshly collected *M. cicadina (Mc)*- and *M. platypediae (Mp)*-infected cicadas, as well as from archived *M. levispora (Ml)*-infected *Okanagana rimosa, M. cicadina* and *M. platypediae* (table S2). Morphology of conidia were studied in 15, 14, and 8 specimens of *M. cicadina*, *M. platypediae*, and *M. levispora,* respectively, while morphology of resting spores was examined in 12, 1 and 1 specimens (table S2). The conidial specimens of *M. cicadina*-infected periodical cicadas spanned ten Broods and six of the seven *Magicicada* species collected over 38 years and distributed across the eastern U.S., while *M. platypediae*- and *M. levispora*-infected cicadas were from a single populations collected in New Mexico (2017) and Michigan (1998), USA, respectively (table S2).

Mean conidia (*Mc* = 14.76 µm x 16.65 µm and *Ml* = 8.78 µm x 14.26 µm) and resting spore (*Mc* = 42.42 µm and *Ml* = 40.7 µm) dimensions for *M. cicadina* and *M. levispora* overlapped with previously reported measurements (fig. S1). In contrast, spore dimensions in the majority of *M. platypediae* specimens fell completely outside the reported range for this species (table S2). Interestingly, all the *M. platypediae* measurements fell within the reported range for *M. levispora,* including a single resting spore specimen, which had not been previously reported for *M. platypediae* (fig. S1, table S2).

Molecular and Phylogenetic studies

Molecular and phylogentic studies were conducted to complement morphological studies and help provide species-level identification of collected specimens. *Massospora* spp., prior to this study, were represented in NCBI Genbank by four DNA sequences from a single isolate of *M. cicadina* (ARSEF 374; NCBI Genbank accessions EF392377, EF392548, EF392492, DQ177436). GenBank blastn results of LSU sequences from *M. cicadina, M. levispora, and M. platypediae* DNA templates were 86-88% identical to *Eryniopsis caroliniana* strain ARSEF 640 (EF392387), but only 82-84% identical to *Massospora cicadina* strain ARSEF 374. Independent DNA extraction and sequencing of ARSEF 374 fungal plug tissue failed to generate a high

quality LSU sequence and therefore it was not included in the LSU phylogeny. GenBank blastn results of SSU sequences from *M. cicadina, M. levispora*, and *M. platypediae* DNA templates were 96-97% identical to *Entomophthora schizophora* (AF052402) and *Entomophthora thripidum* (AF296755). Despite a deposited SSU reference sequence for *M. cicadina*, none of the *Massospora* species sequenced in this study had a reportable BLAST hit to *M. cicadina* ARSEF 374. In contrast, successful amplification of SSU from the resampled ARSEF 374 specimen by our lab did yield a usable sequence 100% identical to other *M. cicadina* strains from this study using pairwise BLAST searches and 97% identical to strains of *E. schizophora* and *E. thripidum*. GenBank blastn results of EFL sequences, an elongation factor like protein present in some early diverging fungi (*1*), from *M. levispora* and *M. platypediae* DNA templates were both 83% identical to *Entomophthora muscae* isolate AFTOL-ID 28 (DQ275343), while *M. cicadina* was 81% identical to *Geranomyces variabilis* voucher JEL379 (HQ901615). Attempts were made to extract and amplify DNA from type specimens of *M. tettigatis* (53297), *M. levispora* (53294), *M. diceroproctae* (53305), and *M. cicadettae* (CUP-060126) but were unsuccessful using Sanger sequencing.

Phylogenetic analysis of the individual partitions (not shown) and combined dataset (LSU+SSU) using maximum likelihood (ML) resolved all sampled *Massospora* in a strongly supported monophyletic group sister to a clade containing *Entomophthora* and *Arthrophaga* (fig. S2). EFL was excluded from the concatenated dataset on account of its punctate distribution across the Entompohtorales (*1*). *Massospora cicadina* formed a single lineage that encompassed all sampled broods and *Magicicada* species with the exception of previously deposited *M. cicadina* ARSEF 374, which aligned with *Entomophthora*. Surprisingly, *Massospora levispora* and *M. platypediae* were not resolved as genealogically exclusive species. Instead, they grouped together in a single monophyletic clade clonal lineage sister to *M. cicadina* (fig. S2). Coupled with the morphological data, we propose two possible scenarios: 1) a single annual-cicada infecting *Massospora* species, which includes *M. levispora* and *M. platypediae,* encompasses a broader geographic and host range than previously reported, or 2) *M. levispora*, in addition to *M. platypediae,* can infect wing-banger cicadas. Given that more data is needed to support these hypotheses, each of the three *Massospora* species, *M. cicadina*, *M. platypediae*, and *M. levispora*, will be maintained for clarity.

ML bootstrap analysis of the individual LSU and SSU partitions (data not shown) revealed strong support for two *Massospora* species although a polytomy in the SSU tree could not fully resolve the relationships among *Massospora* spp., a clade containing *Entomophthora* and a fourth clade containing *Arthrophaga myriapodina* and *Eryniopsis ptycopterea*.

Reference LSU, SSU, and EFL DNA sequences for *M. cicadina*, *M. platypediae,* and *M. levispora* were deposited in NCBI Genbank (MH483015 - MH483011 and MH547099).

Other alkaloids detected using global metabolomics

Using global metabolomics, other tryptamine-containing natural products were identified from metabolomics databases for both *M. cicadina* and *M. platypediae* at significantly higher levels than the cicada controls. In *M. platypediae*, two lysergic acid pathway product intermediates, 4-Dimethylallyl-L-tryptophan (4-DMAT) and agroclavine (ergot alkaloid), were observed at 17.6x and 16.8x log fold change higher than in *M. cicadina* and in the cicada control, respectively. In *M. cicadina* and *M. platypediae*, aspidospermine, another tryptamine-containing alkaloid, was 16.8x and 14.5x log fold change higher compared to the cicada control. Additionally, Nb-lignoceroyltryptamine was also recovered from *M. cicadina* and was 16.8x log fold change higher than *M. platypediae* and the negative control. Slaframine, a piperidine alkaloid, was also recovered from *M. cicadina* at 13.6x log fold change increase over *M. platypediae* and the control. However, unlike cathinone, psilocybin, and psilocin, none of these other alkaloids were confirmed via fragmentation. Furthermore, analysis using LC/UV for agroclavine failed to confirm its presence. Together these results indicate that these IDs are incorrect and resulted from isobaric compounds (having the same m/z as a previously characterized compound).

Detection of Cathinone from *M. cicadina* resting spores

Results from a second global metabolomics study only comparing resting spore versus conidial isolates of *M. cicadina* showed a more complex metabolome from resting spore isolates (fig S4). Of the 213 metabolites shown to be significantly different between conidia and resting spores ($p<0.05$) and have a log fold change >2.5, only 31 metabolites were shown to be upregulated in the conidia, including cathinone which showed a 4.61 ± 0.28 higher fold change compared to its resting spore counterpart. However, none of the cathinone pathway intermediates

were detected. Further studies are needed to clarify the role of cathinone in secondary resting spore infections.

Detection of Cathinone CoA Dependent Pathway intermediates

Over 10 CoA species were monitored including all five hypothesized cathinone CoA-dependent pathway intermediates, Trans-cinnamoyl-CoA, 3-Hydroxy-3-phenylpropionyl-CoA, 3-oxy-3-phenylpropionyl-CoA, benzoyl-CoA. The most abundant CoAs detected were Acetyl-CoA and Malonyl-CoA with other CoAs detected with varying intensities across pooled samples. However, none of the cathinone CoA-dependent pathway intermediates monitored were detected, meaning they were not present or below detectable limits. Given the labile nature of amphetamines (*2*) and the nearly 2-year delay in assessing these samples for these pathway specific intermediates, additional freshly collected samples should help clarify these findings.

LC-MS/MS absolute quantification of psilocin

Following the detection of psilocybin from *M. platypediae* using global metabolomics, we sought to quantify this compound from individual fungal plug extracts from all three cicada-fungal pairs including *M. levispora*-infected annual cicadas, which was shown to from a single monophyletic clonal lineage with *M. platypediae*. *Massospora levispora* was excluded from global metabolomics studies due to long-term storage of specimens in ethanol that prevented proper normalization but was still suitable for absolute quantitation. Despite its detection in all *M. platypediae* conidial specimens (table S1), attempts to quantify psilocybin revealed it was below limit of quantification. Given these results we next sought to quantify psilocin, the biologically active form of psilocybin, as this metabolite can be obtained by dephosphorylation of natural psilocybin under strongly acidic or under alkaline conditions. After finding quantifiable amounts of psilocin from *M. platypediae*-infected annual cicadas, we monitored for both compounds in the remaining samples.

LC-MS/MS absolute quantification of monoamine alkaloids from healthy cicadas

To confirm the fungal origin of monoamine alkaloids from *Massospora*-infected cicadas, we performed targeted LC-MS/MS absolute quantification of cathinone, psilocybin and psilocin on independent populations of the two cicada hosts included in the global metabolomics study, *Platypedia putnami* from CA and Brood VII *Magicicada septendecim* from NY. Sampling included five outwardly asymptomatic males and females for each cicada species. Similar to cicada controls used in the global metabolomics, results of these studies failed to detect any of the three monitored compounds from macerated posterior abdominal sections (table S4).

PCR for *Psilocybe* Psi genes

Deposited nucleotide sequences for four psilocybin biosynthesis enzymes (*3*), PsiD (l-tryptophan decarboxylases, PsiK (4-hydroxytryptamine kinase), PsiM (norbaeocystin methyltransferase), and PsiH (a monooxygenase), described from hallucinogenic mushrooms, *Psilocybe cubensis* and *Psilocybe cyanescens*, were used to develop eight degenerate primer sets in attempt to amplify P*silocybe* psilocybin biosynthesis genes in *Massospora platypediae*. *Psilocybe cubense* genomic DNA served as a positive control in all PCR reactions. All primer sets failed to amplify Psi-specific PCR targets in any of the assayed *Massospora* DNA templates but three primer sets, one for PsiD, PsiM and PsiK, each yielded amplicons of expected size for the *Psilocybe cubense* control, validating the primer design against psilocybin biosynthesis genes. GenBank blastn results of PsiD, PsiM and PsiK sequences from *Psilocybe* DNA templates were 98-99% identical to *Psilocybe cubensis* strain FSU 12409 (KY984101, KY984100 and KY984099, respectively). All three sequences were deposited in NCBI Genbank (MH483012 - MH483014).

Conidiobolus coronatus pathogenicity assays for alkaloid detection

To test functionality of this single locus uncovered in *Conidiobolus coronatus* (Entomophthorales) during queries for evidence of psilocybin gene clustering among homologs of the candidate sequences, eight representative *C. coronatus* isolates spanning two insect orders (Hemiptera and Thysanoptera), Basidiomycotan fungi, and a horse (table S12) were acquired from the Agricultural Research Service Collection of Entomopathogenic Fungal Cultures

(ARSEF) chosen for live-plating and injection entomopathogenicity assays on *Galleria mellonella* (*4*). By 24-hours post-inoculation, all inoculated individuals were dead and by 48-hours all live plated individuals were symptomatic with many dead larvae. Symptomatic individuals were collected at 48-hour post-inoculation and immediately placed at -80 C prior to maceration to permit targeted LC-MS/MS absolute quantification of psilocybin and psilocin targeted metabolomics from both infected larvae and pure cultures of each isolate. Results of these studies failed to detect either compound from macerated infected larvae or week-old fungal cultures of these same isolates on Sabouraud Dextrose Yeast Agar. However, *C. coronatus* isolate ARSEF 8715 (NRRL 28638), from which the genome and candidate locus was obtained, was not available and therefore was not included in the study.

Parsimonious reconstructions of active host transmission in Entomophthoralean fungi

Any topology that resolves the clade containing *Massospora* and *Entomophthora* sister to *Strongwellsea* requires either two independent gains of active transmission behavior, one of which is followed by a single loss, or a single gain in the ancestral progenitor followed by three losses, the latter of which suggests this behavior modification may have been the key innovation that led to diversification of the Entomphthoralean fungi. Active host transmission is known from four of some 20 Entomophthoralean genera (*5*,*6*), two of which, *Entomophthora* and *Entomphaga*, also include well known examples of summit behavior. Presumed delays in *Massospora*-induced mortality require further confirmation as do their cause(s) which may include changes in: 1) behavioral heliothermy, the basic thermoregulatory pattern in cicadas (*7*) which may restrict pathogen growth inside the host (*8*); 2) pathogen virulence including the expansion or contraction of hydrolytic enzymes, such as chitinases, proteases and lipases (*9*); 3) other extrinsic biotic and abiotic factors (*8*); or 4) a combination of these factors.

Female-typical courtship behaviors by male *Massospora*-infected *P. putnami* and the possible hormonal changes driving these behaviors

Activity-level effects of psilocybin may provide a plausible explanation of the addition of female-typical behaviors to the repertoires of male *Massospora*-infected wing-banger cicadas.

Infected males were found to produce significantly fewer crepitations (wing tapping required for mating) per observation period (*10*), a female-typical behavior, possibly resulting from impaired temporal processing, a known effect of psilocybin in vertebrates (*11*). However, the atypical mating behaviors of other cicada species may be better explained by *Massospora*-mediated hormonal changes incidental to amphetamine and psilocybin production (fig. S5*)*. For example, 2-Hydroxyestradiol, a catechol estrogen and metabolite of estradiol and 3-O-acetylecdysone 2-phosphate, a metabolite of ecdysone, a steroidal prohormone of the major insect molting hormone 20-hydroxyecdysone, were both found to be upregulated in male *M. platypediae* compared to a mixed sex group of *M. cicadina* and healthy controls using global metabolomics. These hormones may accumulate as a result of disruption of typical pathways (i.e. castration of testis) or they may be fungus-derived.

Testing for facultative plant associations in *M. cicadina*

The root colonizing properties of *Massospora* on sudangrass (*Sorghum sudanense*), a model system for arbuscular mycorrhizal fungi (https://invam.wvu.edu/methods/cultures/host-plant-choices) were assessed following the discovery of a plant-based metabolite, cathinone, in *Massospora*. Ten plants were inoculated with 300 *M. cicadina* resting spores, identical to inoculations using AMF spores. After five months, plants were harvested and roots washed to confirm whether root colonization had taken place. Root staining of four randomly chosen plants failed to confirm a facultative mutualism with plants.

Supplementary Figures

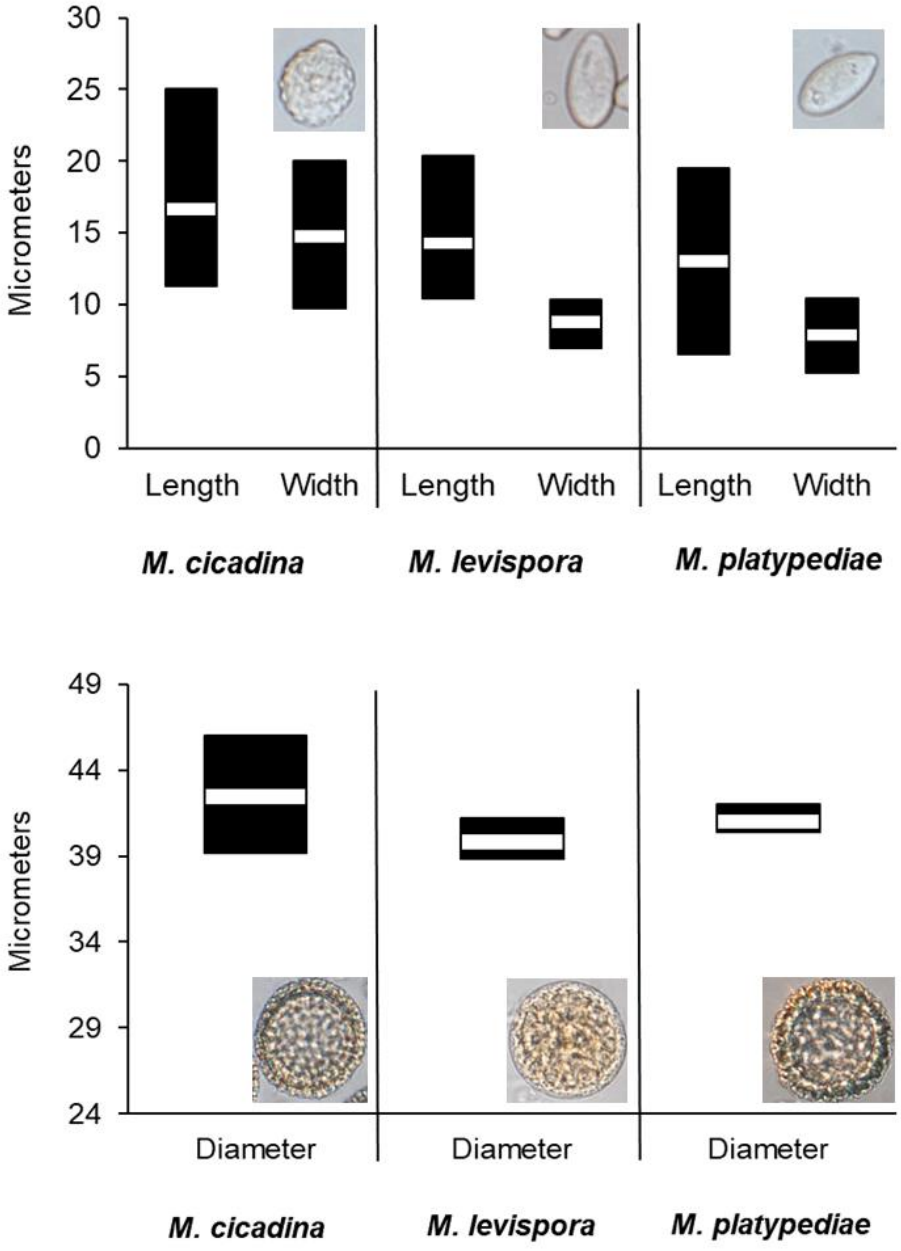


Fig. S1. Mean (A) conidia and (B) resting spore dimensions for three *Massospora* species sampled from infected cicadas. White bars indicate overall mean values while black bars indicate the range of variation in spore dimensions. Twenty-five conidia or resting spores were measured for each specimen except for *M. levispora* and *M. platypediae* resting spores, in which 50 spores were measured. Sampling sizes were as follows: For conidia *Mc* = 15, *Ml* = 8, and *Mp* = 14; For resting spores, *Mc* = 11, *Ml* = 1, *Mp* = 1.

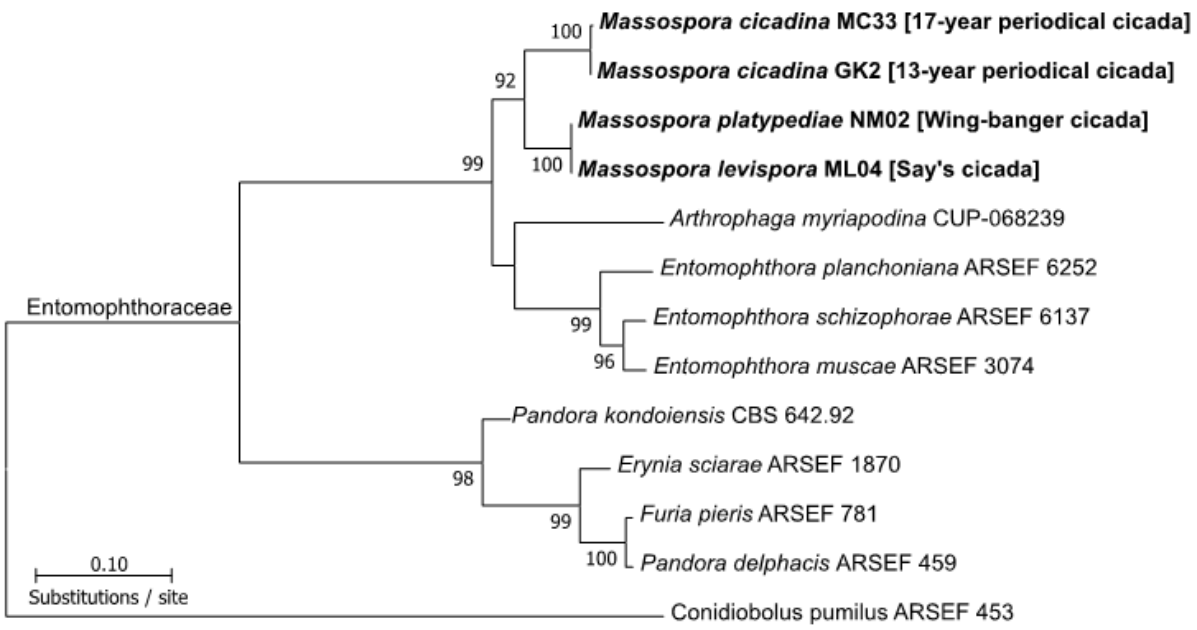


Fig. S2. Concatenated LSU+SSU maximum likelihood (ML) tree consisting of *Massospora* species and related species in the Entomophthorales. **B**ootstrap support is indicated near each node and only values greater than 70% are shown.

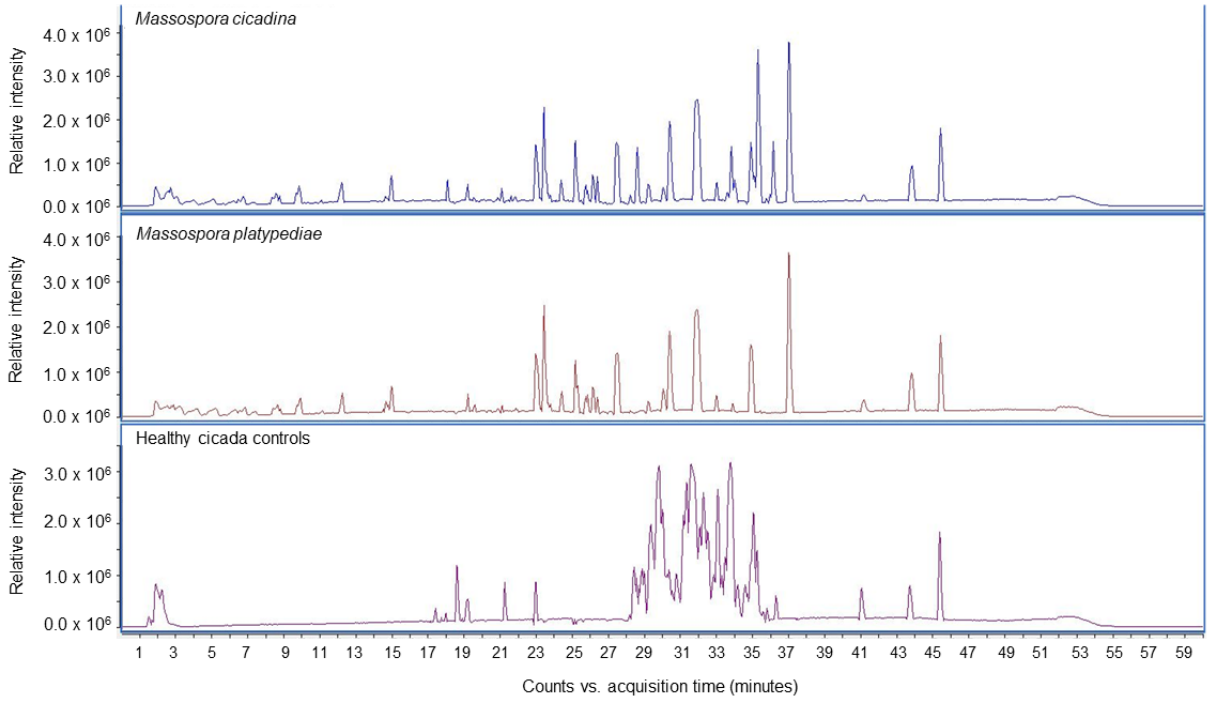


Fig. S3. Representative total base peak chromatograms (BPC) from the global metabolomics analysis of *M. cicadina*, *M. platypediae*, and a healthy cicada controls.

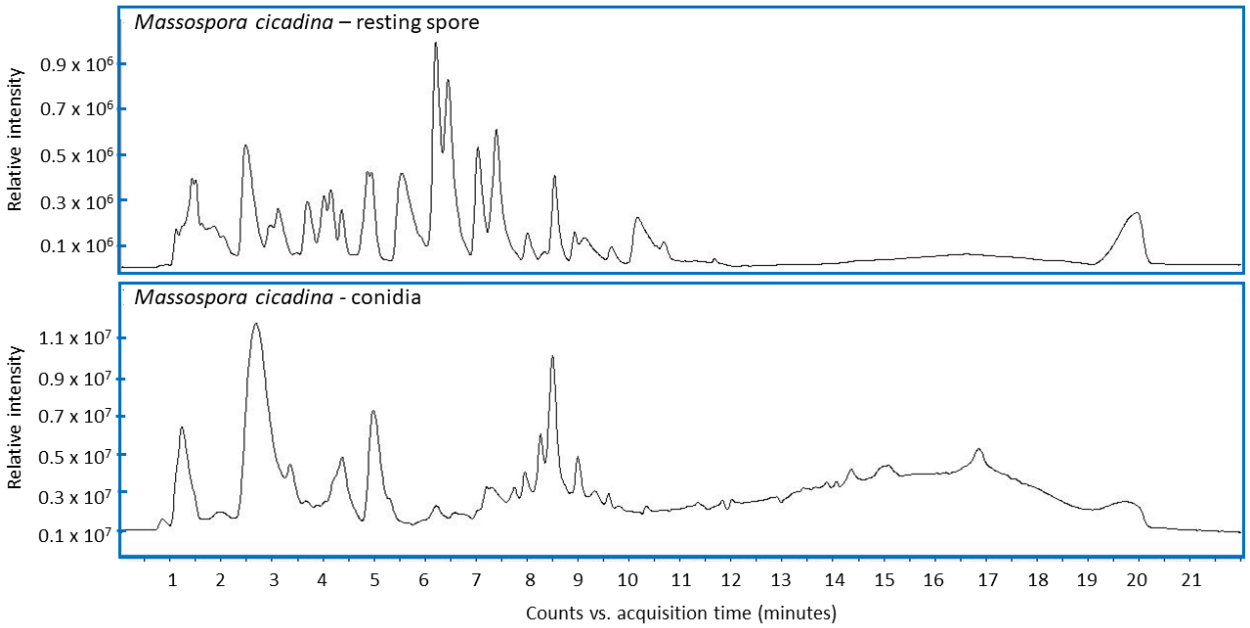
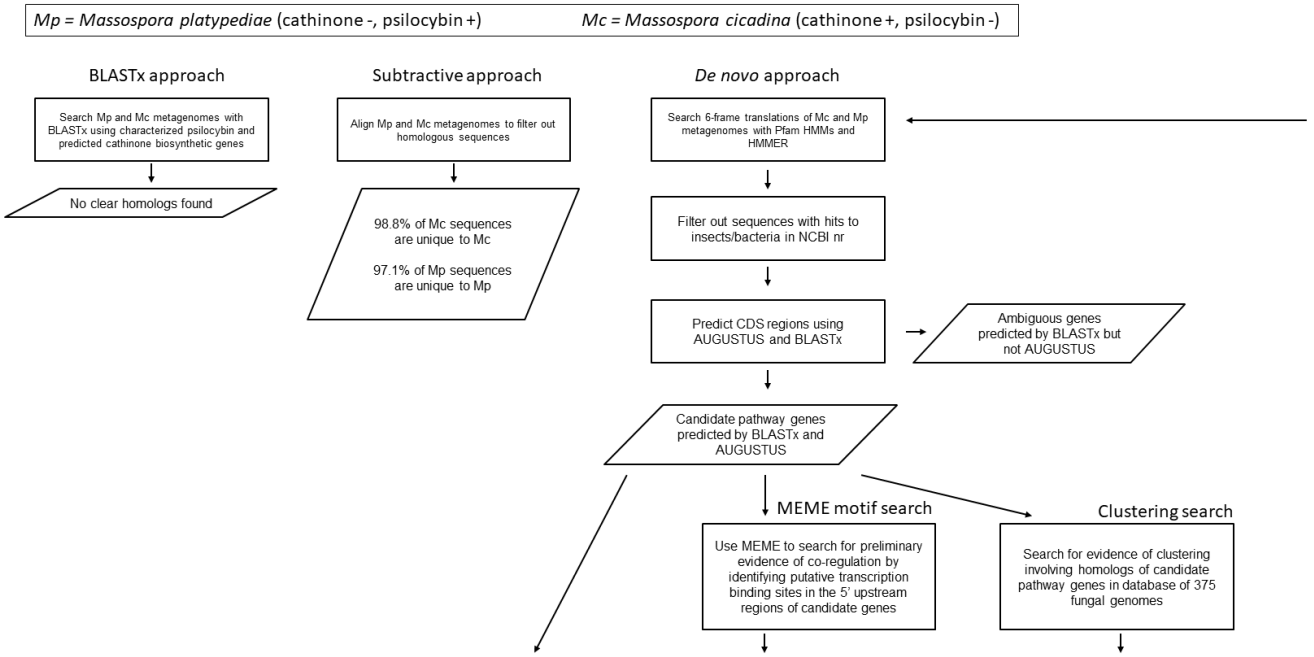


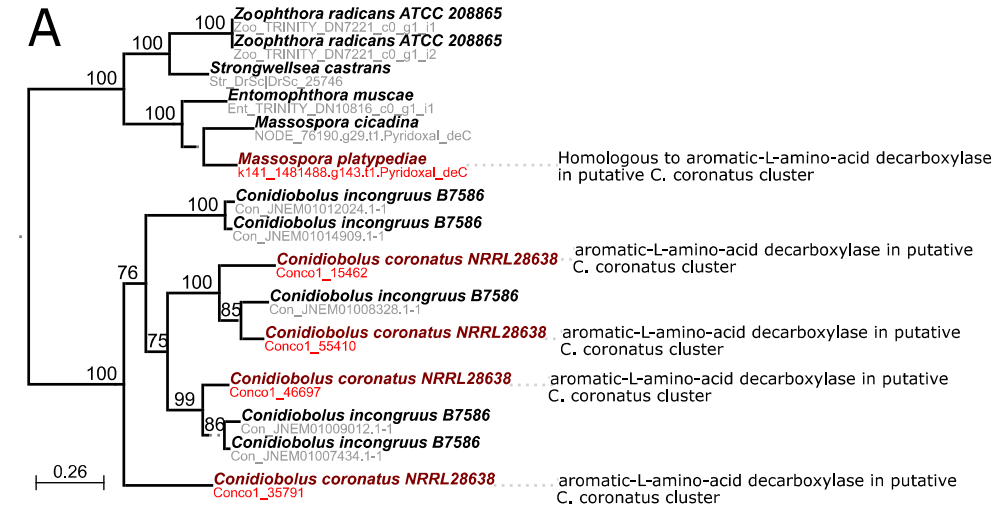
Fig. S4. Representative total base peak chromatograms (BPC) from the global metabolomics analysis of *M. cicadina* resting spores versus *M. cicadina* conidia.

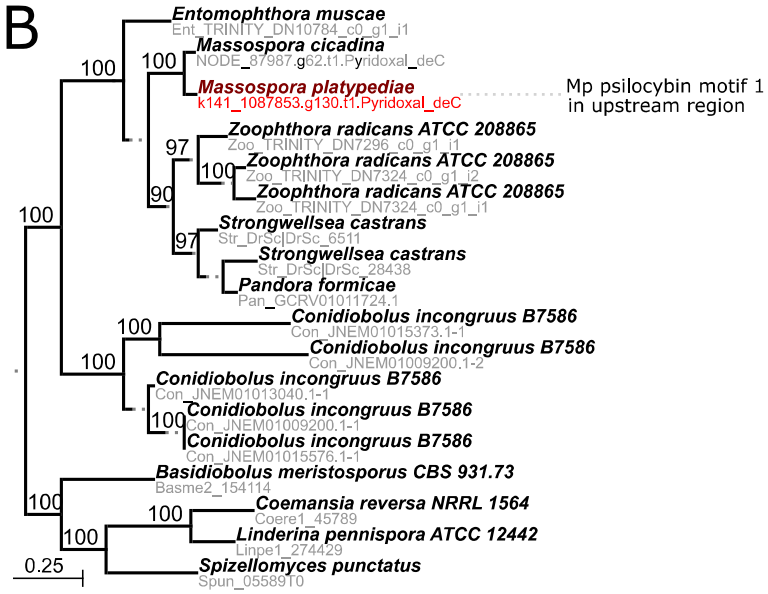


Pathway	Predicted gene function	Number of Pfam HMMs
Psilocybin	decarboxylase	3
	methyltransferase	33
	phosphorylase	1
	hydroxylase	1
Cathinone	Ammonia lyase	1
	Co-A ligase	1
	hydratase	1
	dehydrogenase	2
	thiolase	1
	carboligase	1
	transaminase	1
	reductase	2

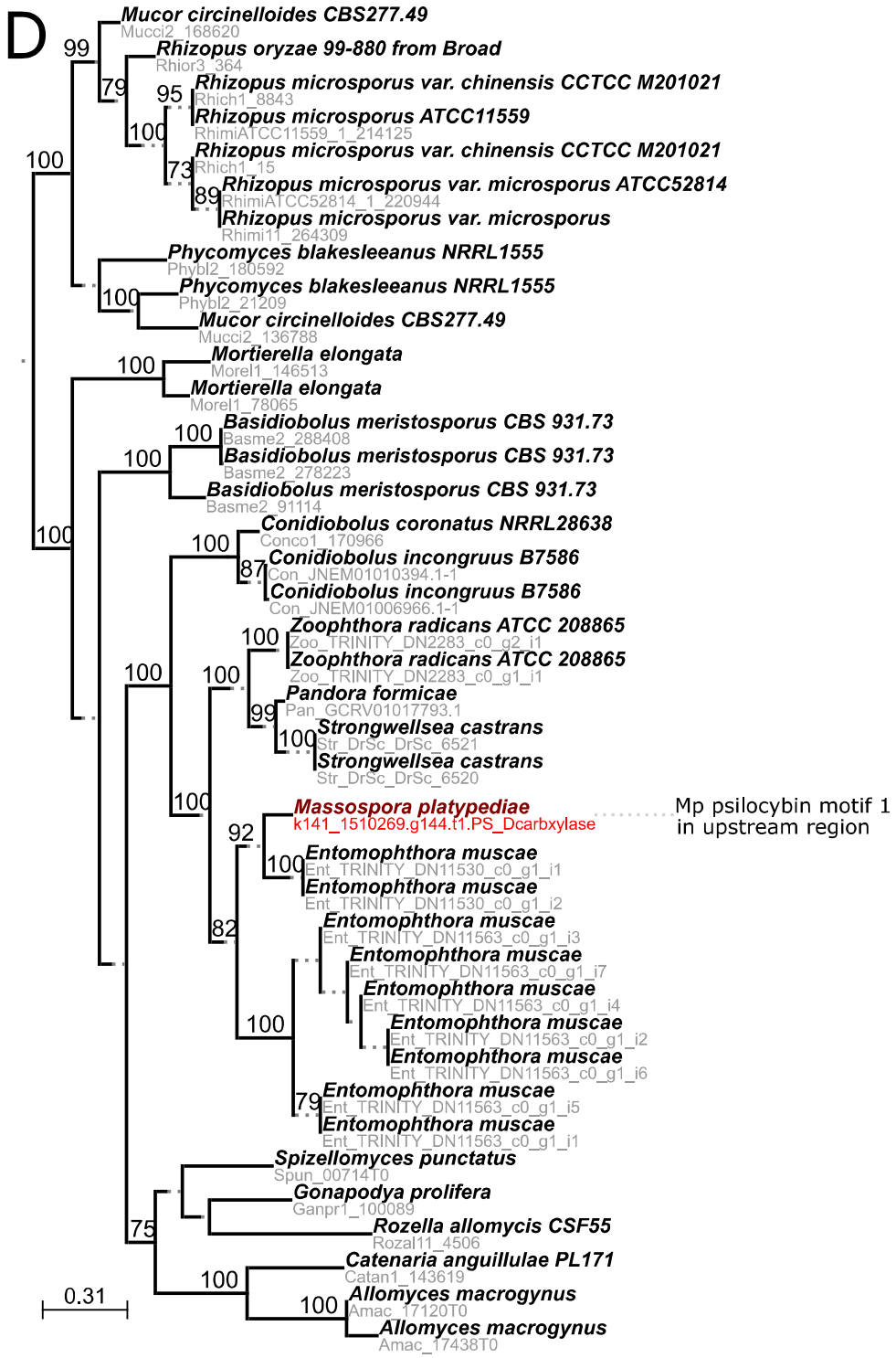
Pathway	**Gene function**	**# of candidate genes** *Mp*	**# of candidate genes** *Mc*	**# of gene homolog groups (# of genes in groups)** *Mp* unique	*Mc* unique	Both	**MEME motif search** *Mp*	*Mc*	**Clustering search**
Psilocybin	decarboxylase	10	5	4 (4)	0 (0)	4 (11)	5 motifs total. 2 motifs present upstream of methyltransferase candidates	1 motif total. No motifs present upstream of methyltransferase candidates	309 clusters found. 123 clusters contain homologs of methyltransferase candidates. 1 notable cluster found in *Conidiobolus coronatus* (Entomophthorales)
	methyltransferase	23	21	13 (14)	12 (12)	9 (18)			
	phosphorylase	0	0	0 (0)	0 (0)	0 (0)			
	hydroxylase	36	23	8 (12)	5 (5)	10 (42)			
Cathinone	Ammonia lyase	0	0	0 (0)	0 (0)	0 (0)	7 motifs total. 2 motifs present upstream of transaminase candidates.	2 motifs total. Both motifs are found upstream of the same hydratase, reductase and transaminase candidates.	1548 clusters found. No clusters contain homologs of transaminase candidates.
	Co-A ligase	15	11	6 (6)	6 (6)	4 (14)			
	hydratase	2	2	1 (1)	1 (1)	1 (2)			
	dehydrogenase	10	5	8 (8)	3 (3)	2 (4)			
	thiolase	4	3	1 (1)	1 (1)	2 (5)			
	carboligase	2	1	1 (1)	0 (0)	1 (2)			
	transaminase	11	11	5 (5)	6 (6)	5 (11)			
	reductase	20	14	10 (10)	4 (4)	8 (20)			

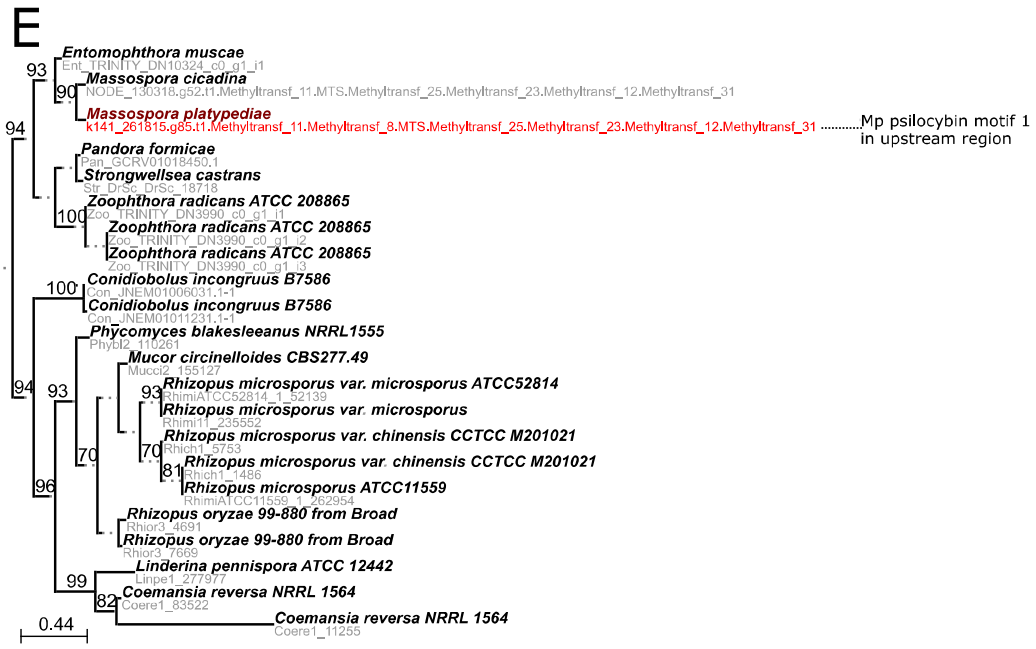
Fig. S5. Psilocybin and Cathinone biosynthesis pathway gene discovery pipeline.

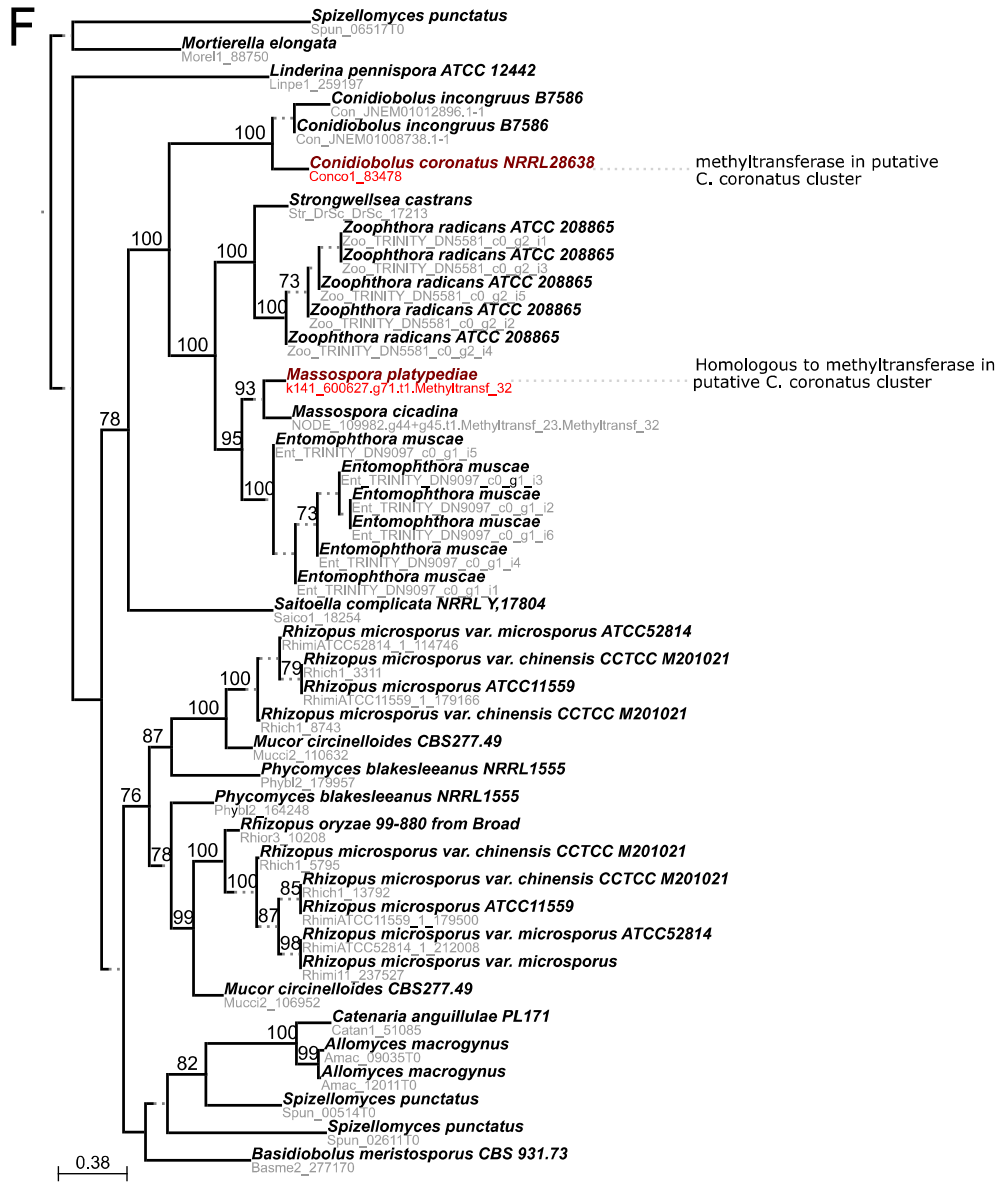

A
Zoophthora radicans ATCC 208865
Zoo_TRINITY_DN7221_c0_g1_i1
Zoophthora radicans ATCC 208865
Zoo_TRINITY_DN7221_c0_g1_i2
Strongwellsea castrans
Str_DrSc|DrSc_25746
Entomophthora muscae
Ent_TRINITY_DN10816_c0_g1_i1
Massospora cicadina
NODE_76190.g29.t1.Pyridoxal_deC
Massospora platypediae
k141_1481488.g143.t1.PyridoxaL_deC
Homologous to aromatic-L-amino-acid decarboxylase in putative C. coronatus cluster
Conidiobolus incongruus B7586
Con_JNEM01012024.1-1
Conidiobolus incongruus B7586
Con_JNEM01014909.1-1
Conidiobolus coronatus NRRL28638
Conco1_15462
aromatic-L-amino-acid decarboxylase in putative C. coronatus cluster
Conidiobolus incongruus B7586
Con_JNEM01008328.1-1
Conidiobolus coronatus NRRL28638
Conco1_55410
aromatic-L-amino-acid decarboxylase in putative C. coronatus cluster
Conidiobolus coronatus NRRL28638
Conco1_46697
aromatic-L-amino-acid decarboxylase in putative C. coronatus cluster
Conidiobolus incongruus B7586
Con_JNEM01009012.1-1
Conidiobolus incongruus B7586
Con_JNEM01007434.1-1
0.26
Conidiobolus coronatus NRRL28638
Conco1_35791
aromatic-L-amino-acid decarboxylase in putative C. coronatus cluster

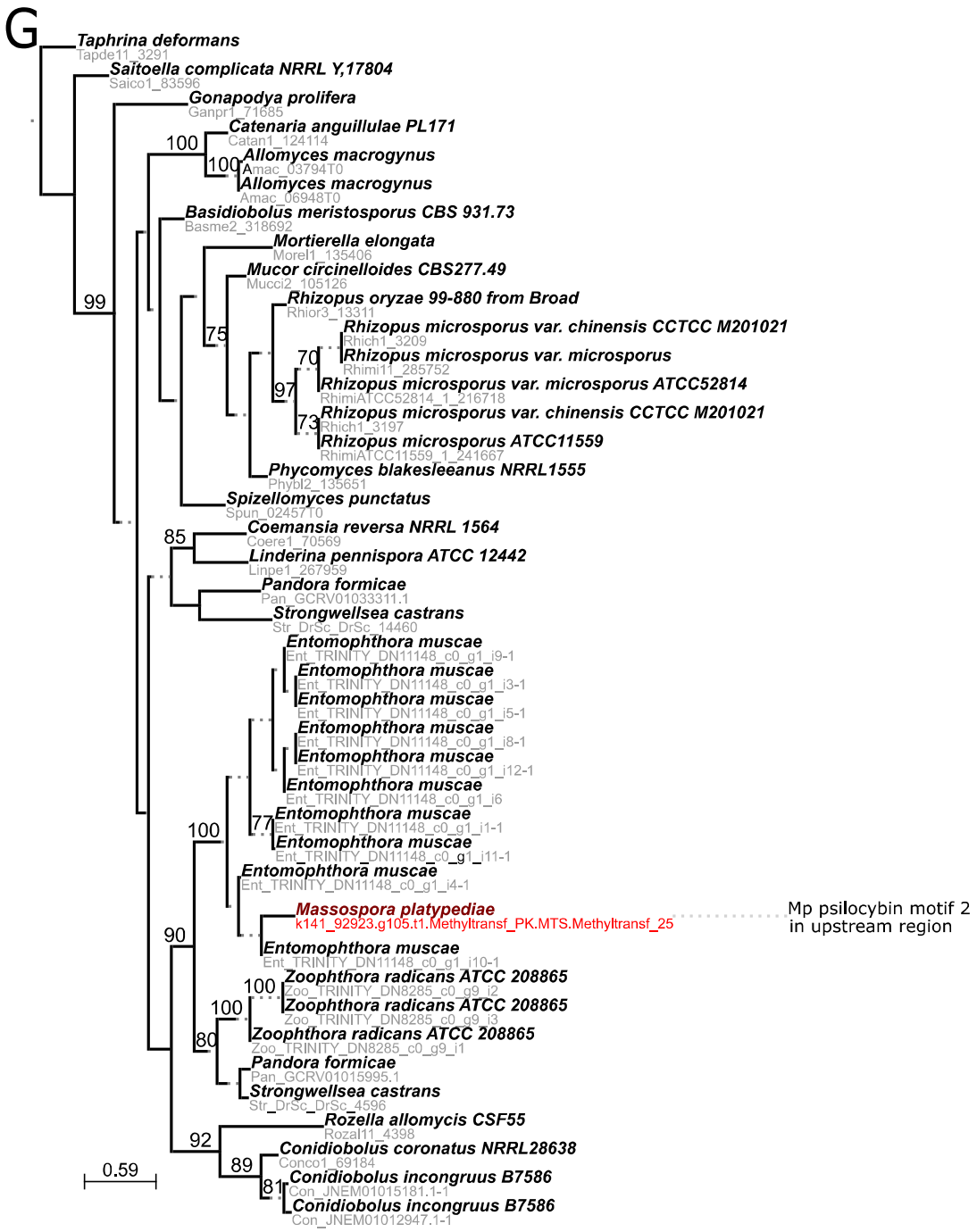

B
Entomophthora muscae
Ent_TRINITY_DN10784_c0_g1_i1
Massospora cicadina
NODE_87987.g62.t1.Pyridoxal_deC
Massospora platypediae
k141_1087853.g130.t1.Pyridoxal_deC
Mp psilocybin motif 1 in upstream region
Zoophthora radicans ATCC 208865
Zoo_TRINITY_DN7296_c0_g1_i1
Zoophthora radicans ATCC 208865
Zoo_TRINITY_DN7324_c0_g1_i2
Zoophthora radicans ATCC 208865
Zoo_TRINITY_DN7324_c0_g1_i1
Strongwellsea castrans
Str_DrSc|DrSc_6511
Strongwellsea castrans
Str_DrSc|DrSc_28438
Pandora formicae
Pan_GCRV01011724.1
Conidiobolus incongruus B7586
Con_JNEM01015373.1-1
Conidiobolus incongruus B7586
Con_JNEM01009200.1-2
Conidiobolus incongruus B7586
Con_JNEM01013040.1-1
Conidiobolus incongruus B7586
Con_JNEM01009200.1-1
Conidiobolus incongruus B7586
Con_JNEM01015576.1-1
Basidiobolus meristosporus CBS 931.73
Basme2_154114
Coemansia reversa NRRL 1564
Coere1_45789
Linderina pennispora ATCC 12442
Linpe1_274429
0.25
Spizellomyces punctatus
Spun_0558910

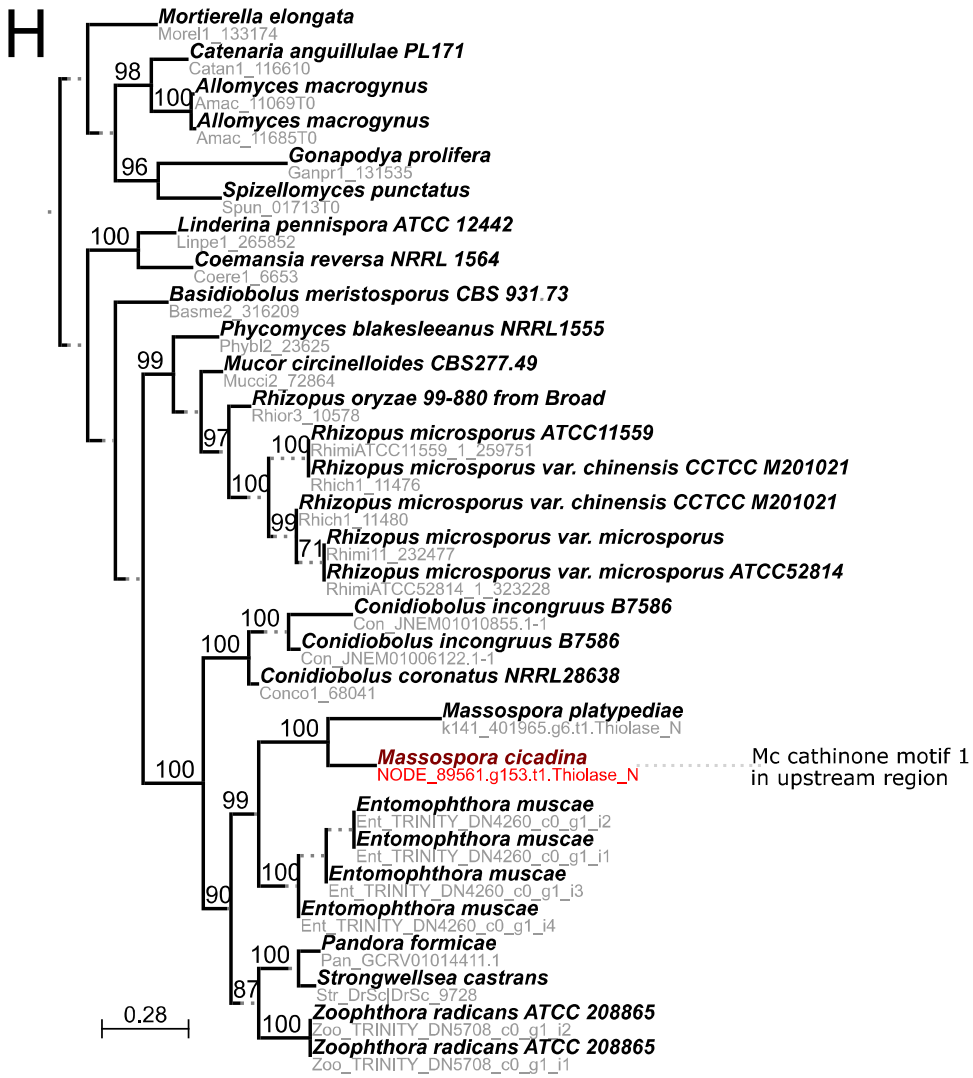

C
Basidiobolus meristosporus CBS 931.73
Basme2_341389
Basidiobolus meristosporus CBS 931.73
Basme2_240833
Basidiobolus meristosporus CBS 931.73
Basme2_224980
Phycomyces blakesleeanus NRRL1555
Phybl2_98182
Mucor circinelloides CBS277.49
Mucci2_106396
Rhizopus oryzae 99-880 from Broad
Rhior3_9845
Rhizopus microsporus var. chinensis CCTCC M201021
Rhich1_9029
Rhizopus microsporus ATCC11559
RhimiATCC11559_1_197324
Rhizopus microsporus var. chinensis CCTCC M201021
Rhich1_8209
Rhizopus microsporus var. microsporus
Rhimi11_278875
Rhizopus microsporus var. microsporus ATCC52814
RhimiATCC52814_1_236878
Mortierella elongata
Morel1_20807
Catenaria anguillulae PL171
Catan1_60478
Allomyces macrogynus
Amac_16281T0
Allomyces macrogynus
Amac_08932T0
Linderina pennispora ATCC 12442
Linpe1_267640
Coemansia reversa NRRL 1564
Coere1_78655
Conidiobolus incongruus B7586
Con_JNEM01014023.1-1
Conidiobolus incongruus B7586
Con_JNEM01013948.1-1
Conidiobolus coronatus NRRL28638
Conco1_17131
Conidiobolus coronatus NRRL28638
Conco1_57770
Massospora platypediae
k141_1508870.g136.t1.Pyridoxal_deC
Massospora cicadina
NODE_96706.g42.t1.Pyridoxal_deC
Massospora platypediae
k141_1586431.g106.t1.Pyridoxal_deC
Massospora platypediae
k141_1339227.g137.t1.Pyridoxal_deC
Massospora cicadina
NODE_18769.g11.t1.Pyridoxal_deC
Entomophthora muscae
Ent_TRINITY_DN11622_c0_g1_i3
Entomophthora muscae
Ent_TRINITY_DN11622_c0_g1_i1
Entomophthora muscae
Ent_TRINITY_DN11622_c0_g1_i4
Entomophthora muscae
Ent_TRINITY_DN11622_c0_g1_i2
Zoophthora radicans ATCC 208865
Zoo_TRINITY_DN1429_c0_g1_i1
Zoophthora radicans ATCC 208865
Zoo_TRINITY_DN1429_c0_g2_i1
Pandora formicae
Pan_GCRV01021335.1
Strongwellsea castrans
Str_DrSc|DrSc_5053
0.28
Mp psilocybin motif 1
in upstream region

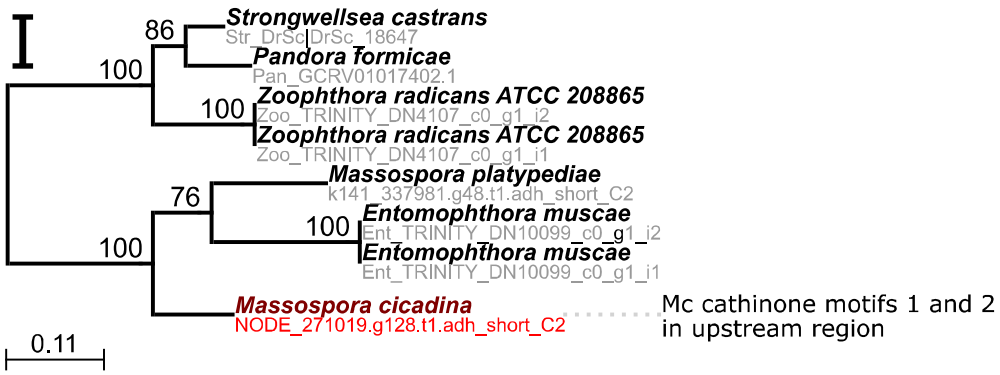

D
Mucor circinelloides CBS277.49
Mucci2_168620
Rhizopus oryzae 99-880 from Broad
Rhior3_364
Rhizopus microsporus var. chinensis CCTCC M201021
Rhich1_8843
Rhizopus microsporus ATCC11559
RhimiATCC11559_1_214125
Rhizopus microsporus var. chinensis CCTCC M201021
Rhich1_15
Rhizopus microsporus var. microsporus ATCC52814
RhimiATCC52814_1_220944
Rhizopus microsporus var. microsporus
Rhimi11_264309
Phycomyces blakesleeanus NRRL1555
Phybl2_180592
Phycomyces blakesleeanus NRRL1555
Phybl2_21209
Mucor circinelloides CBS277.49
Mucci2_136788
Mortierella elongata
Morel1_146513
Mortierella elongata
Morel1_78065
Basidiobolus meristosporus CBS 931.73
Basme2_288408
Basidiobolus meristosporus CBS 931.73
Basme2_278223
Basidiobolus meristosporus CBS 931.73
Basme2_91114
Conidiobolus coronatus NRRL28638
Conco1_170966
Conidiobolus incongruus B7586
Con_JNEM01010394.1-1
Conidiobolus incongruus B7586
Con_JNEM01006966.1-1
Zoophthora radicans ATCC 208865
Zoo_TRINITY_DN2283_c0_g2_i1
Zoophthora radicans ATCC 208865
Zoo_TRINITY_DN2283_c0_g1_i1
Pandora formicae
Pan_GCRV01017793.1
Strongwellsea castrans
Str_DrSc_DrSc_6521
Strongwellsea castrans
Str_DrSc_DrSc_6520
Massospora platypediae
k141_1510269.g144.t1.PS_Dcarbxylase
Mp psilocybin motif 1
in upstream region
Entomophthora muscae
Ent_TRINITY_DN11530_c0_g1_i1
Entomophthora muscae
Ent_TRINITY_DN11530_c0_g1_i2
Entomophthora muscae
Ent_TRINITY_DN11563_c0_g1_i3
Entomophthora muscae
Ent_TRINITY_DN11563_c0_g1_i7
Entomophthora muscae
Ent_TRINITY_DN11563_c0_g1_i4
Entomophthora muscae
Ent_TRINITY_DN11563_c0_g1_i2
Entomophthora muscae
Ent_TRINITY_DN11563_c0_g1_i6
Entomophthora muscae
Ent_TRINITY_DN11563_c0_g1_i5
Entomophthora muscae
Ent_TRINITY_DN11563_c0_g1_i1
Spizellomyces punctatus
Spun_00714T0
Gonapodya prolifera
Ganpr1_100089
Rozella allomycis CSF55
Rozal11_4506
Catenaria anguillulae PL171
Catan1_143619
Allomyces macrogynus
Amac_17120T0
Allomyces macrogynus
Amac_17438T0
0.31

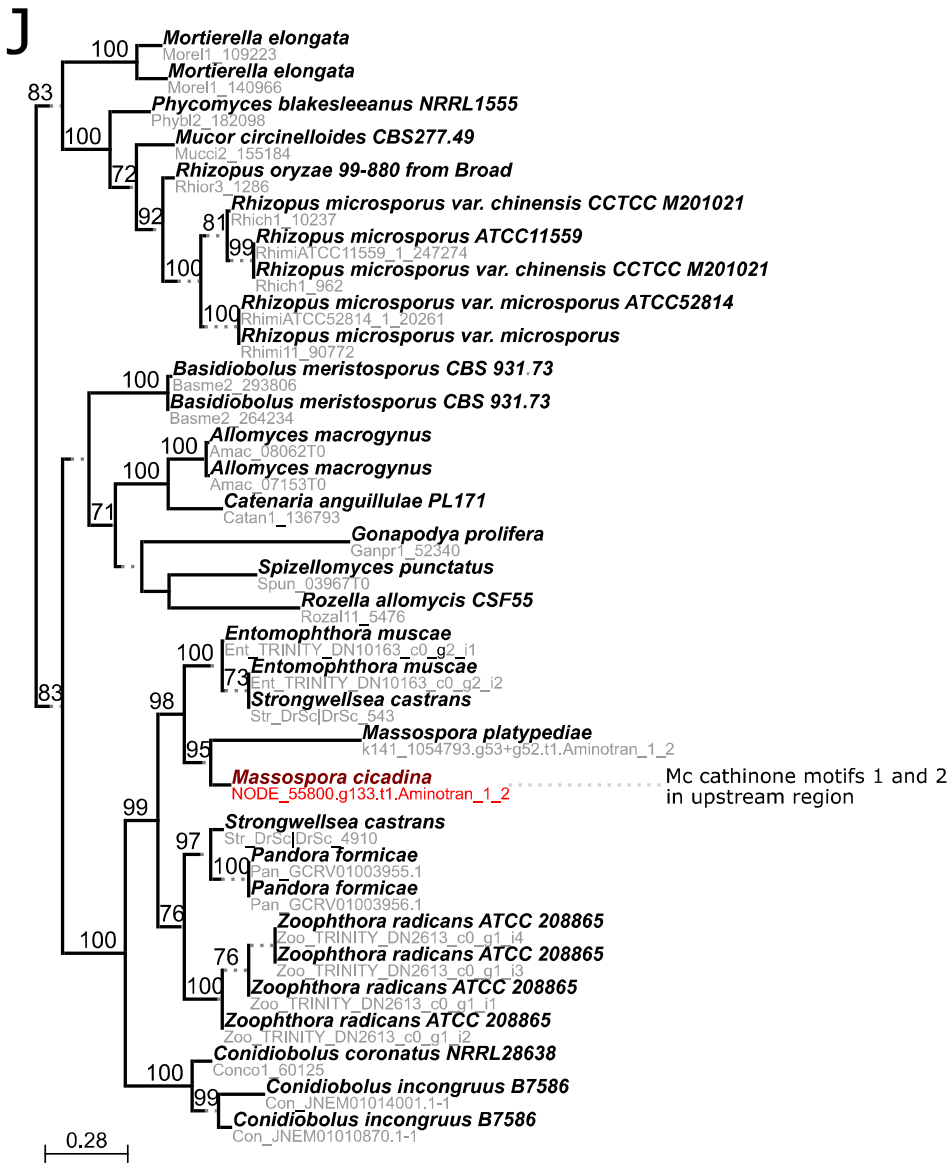

E
Entomophthora muscae
Ent_TRINITY_DN10324_c0_g1_i1
Massospora cicadina
NODE_130318.g52.t1.Methyltransf_11.MTS.Methyltransf_25.Methyltransf_23.Methyltransf_12.Methyltransf_31
Massospora platypediae
k141_261815.g85.t1.Methyltransf_11.Methyltransf_8.MTS.Methyltransf_25.Methyltransf_23.Methyltransf_12.Methyltransf_31
Mp psilocybin motif 1 in upstream region
Pandora formicae
Pan_GCRV010184450.1
Strongwellsea castrans
Str_DrSc_DrSc_18718
Zoophthora radicans ATCC 208865
Zoo_TRINITY_DN3990_c0_g1_i1
Zoophthora radicans ATCC 208865
Zoo_TRINITY_DN3990_c0_g1_i2
Zoophthora radicans ATCC 208865
Zoo_TRINITY_DN3990_c0_g1_i3
Conidiobolus incongruus B7586
Con_INEM01006001.1-1
Conidiobolus incongruus B7586
Con_INEM01011231.1-1
Phycomyces blakesleeanus NRRL1555
PhybI2_110261
Mucor circinelloides CBS277.49
Mucci2_155127
Rhizopus microsporus var. microsporus ATCC52814
RhimiATCC52814_1_52139
Rhizopus microsporus var. microsporus
Rhimi11_235552
Rhizopus microsporus var. chinensis CCTCC M201021
Rhich1_5753
Rhizopus microsporus var. chinensis CCTCC M201021
Rhich1_1486
Rhizopus microsporus ATCC11559
RhimiATCC11559_1_62984
Rhizopus oryzae 99-880 from Broad
Rhior3_4691
Rhizopus oryzae 99-880 from Broad
Rhior3_7669
Linderina pennispora ATCC 12442
Linpe1_277977
Coemansia reversa NRRL 1564
Coere1_83522
Coemansia reversa NRRL 1564
Coere1_11255
0.44

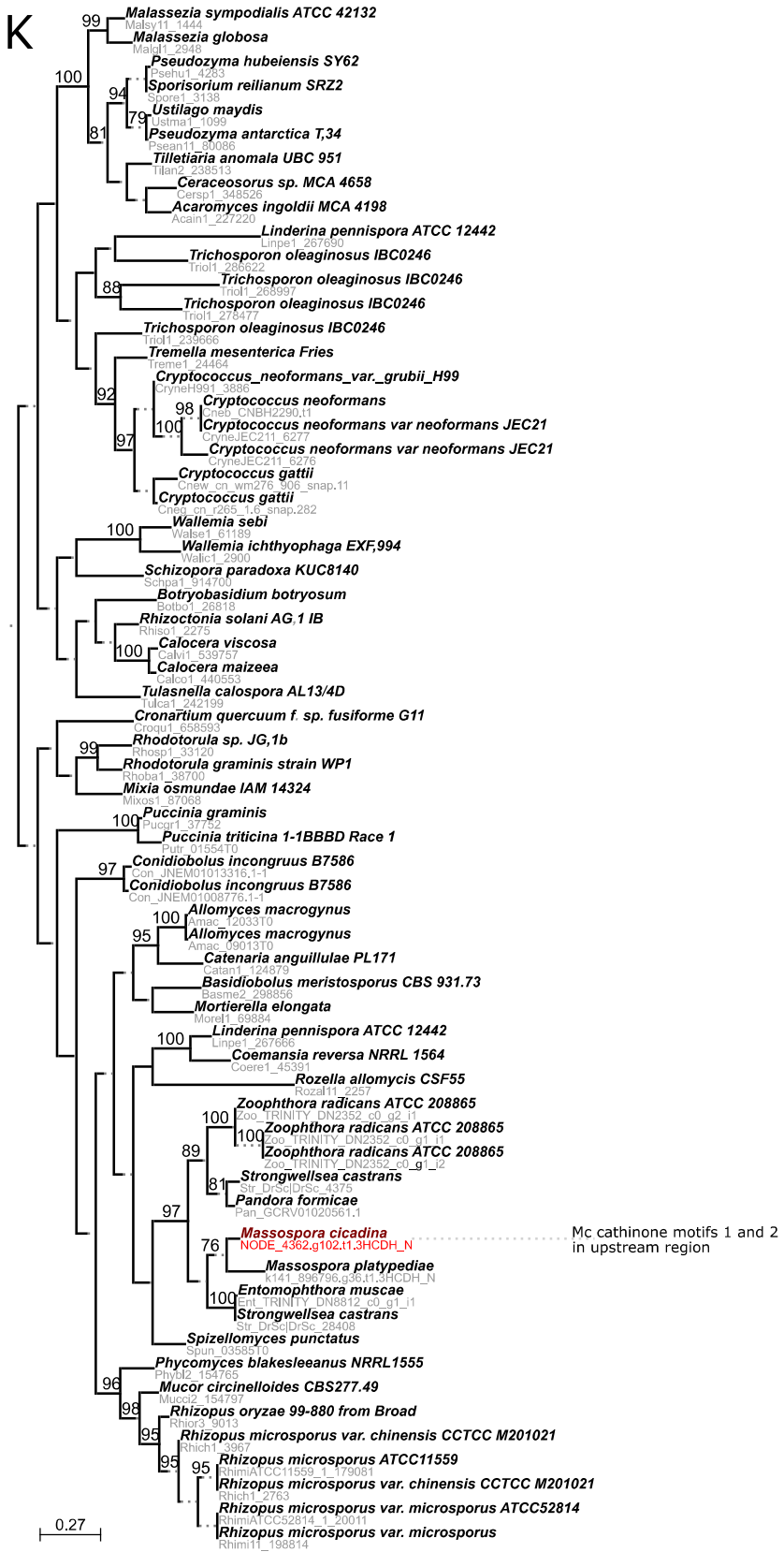

F
Spizellomyces punctatus
Spun_0651710
Mortierella elongata
Morel1_88750
Linderina pennispora ATCC 12442
Linpe1_259197
Conidiobolus incongruus B7586
Con_JNEM01012896.1-1
Conidiobolus incongruus B7586
Con_JNEM01008738.1-1
Conidiobolus coronatus NRRL28638
Conco1_83478
methyltransferase in putative C. coronatus cluster
Strongwellsea castrans
Str_DrSc_DrSc_17213
Zoophthora radicans ATCC 208865
Zoo_TRINITY_DN5581_c0_g2_i1
Zoophthora radicans ATCC 208865
Zoo_TRINITY_DN5581_c0_g2_i3
Zoophthora radicans ATCC 208865
Zoo_TRINITY_DN5581_c0_g2_i5
Zoophthora radicans ATCC 208865
Zoo_TRINITY_DN5581_c0_g2_i2
Zoophthora radicans ATCC 208865
Zoo_TRINITY_DN5581_c0_g2_i4
Massospora platypediae
k141_600627.g71.t1.Methyltransf_32
Homologous to methyltransferase in putative C. coronatus cluster
Massospora cicadina
NODE_109982.g44+g45.t1.Methyltransf_23.Methyltransf_32
Entomophthora muscae
Ent_TRINITY_DN9097_c0_g1_i5
Entomophthora muscae
Ent_TRINITY_DN9097_c0_g1_i3
Entomophthora muscae
Ent_TRINITY_DN9097_c0_g1_i2
Entomophthora muscae
Ent_TRINITY_DN9097_c0_g1_i6
Entomophthora muscae
Ent_TRINITY_DN9097_c0_g1_i4
Entomophthora muscae
Ent_TRINITY_DN9097_c0_g1_i1
Saitoella complicata NRRL Y,17804
Saico1_18254
Rhizopus microsporus var. microsporus ATCC52814
RhimiATCC52814_1_114746
Rhizopus microsporus var. chinensis CCTCC M201021
Rhich1_3311
Rhizopus microsporus ATCC11559
RhimiATCC11559_1_179166
Rhizopus microsporus var. chinensis CCTCC M201021
Rhich1_8743
Mucor circinelloides CBS277.49
Mucci2_110632
Phycomyces blakesleeanus NRRL1555
Phybl2_175957
Phycomyces blakesleeanus NRRL1555
Phybl2_164248
Rhizopus oryzae 99-880 from Broad
Rhior3_10208
Rhizopus microsporus var. chinensis CCTCC M201021
Rhich1_5795
Rhizopus microsporus var. chinensis CCTCC M201021
Rhich1_13792
Rhizopus microsporus ATCC11559
RhimiATCC11559_1_179500
Rhizopus microsporus var. microsporus ATCC52814
RhimiATCC52814_1_212008
Rhizopus microsporus var. microsporus
Rhimi11_237527
Mucor circinelloides CBS277.49
Mucci2_106952
Catenaria anguillulae PL171
Catan1_51085
Allomyces macrogynus
Amac_09035T0
Allomyces macrogynus
Amac_12011T0
Spizellomyces punctatus
Spun_0051410
Spizellomyces punctatus
Spun_0261110
Basidiobolus meristosporus CBS 931.73
Basme2_277170
0.38

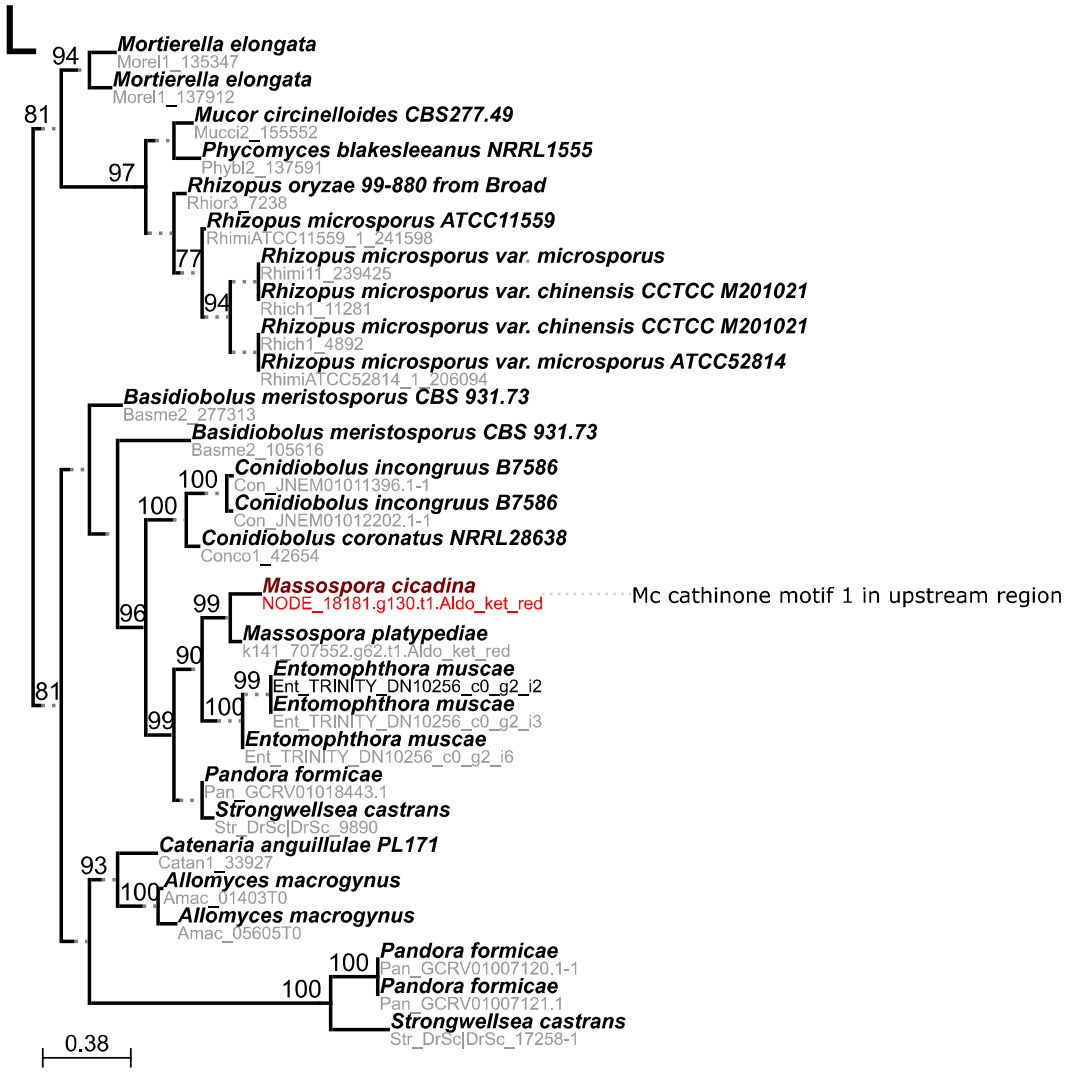

G
Taphrina deformans
Tapde11_3291
Saitoella complicata NRRL Y,17804
Saico1_83596
Gonapodya prolifera
Ganpr1_71685
Catenaria anguillulae PL171
Catan1_124114
Allomyces macrogynus
Amac_03794T0
Allomyces macrogynus
Amac_06948T0
Basidiobolus meristosporus CBS 931.73
Basme2_318692
Mortierella elongata
Morel1_135406
Mucor circinelloides CBS277.49
Mucci2_105126
Rhizopus oryzae 99-880 from Broad
Rhior3_13311
Rhizopus microsporus var. chinensis CCTCC M201021
Rhich1_3209
Rhizopus microsporus var. microsporus
Rhimi11_285752
Rhizopus microsporus var. microsporus ATCC52814
RhimiATCC52814_1_216718
Rhizopus microsporus var. chinensis CCTCC M201021
Rhich1_3197
Rhizopus microsporus ATCC11559
RhimiATCC11559_1_241667
Phycomyces blakesleeanus NRRL1555
Phybl2_135651
Spizellomyces punctatus
Spun_0245T0
Coemansia reversa NRRL 1564
Coere1_70569
Linderina pennispora ATCC 12442
Linpe1_267959
Pandora formicae
Pan_GCRV01033311.1
Strongwellsea castrans
Str_DrSc_DrSc_14460
Entomophthora muscae
Ent_TRINITY_DN11148_c0_g1_i9-1
Entomophthora muscae
Ent_TRINITY_DN11148_c0_g1_i3-1
Entomophthora muscae
Ent_TRINITY_DN11148_c0_g1_i5-1
Entomophthora muscae
Ent_TRINITY_DN11148_c0_g1_i8-1
Entomophthora muscae
Ent_TRINITY_DN11148_c0_g1_i12-1
Entomophthora muscae
Ent_TRINITY_DN11148_c0_g1_i6
Entomophthora muscae
Ent_TRINITY_DN11148_c0_g1_i1-1
Entomophthora muscae
Ent_TRINITY_DN11148_c0_g1_i11-1
Entomophthora muscae
Ent_TRINITY_DN11148_c0_g1_i4-1
Massospora platypediae
k141_92923.g105.t1.MethyltransfPK.MTS.Methyltransf_25
Mp psilocybin motif 2
in upstream region
Entomophthora muscae
Ent_TRINITY_DN11148_c0_g1_i10-1
Zoophthora radicans ATCC 208865
Zoo_TRINITY_DN8285_c0_g9_i2
Zoophthora radicans ATCC 208865
Zoo_TRINITY_DN8285_c0_g9_i3
Zoophthora radicans ATCC 208865
Zoo_TRINITY_DN8285_c0_g9_i1
Pandora formicae
Pan_GCRV01015995.1
Strongwellsea castrans
Str_DrSc_DrSc_4596
Rozella allomycis CSF55
Rozal11_4398
Conidiobolus coronatus NRRL28638
Conco1_69184
Conidiobolus incongruus B7586
Con_JNEM01015181.1-1
Conidiobolus incongruus B7586
Con_JNEM01012947.1-1
0.59

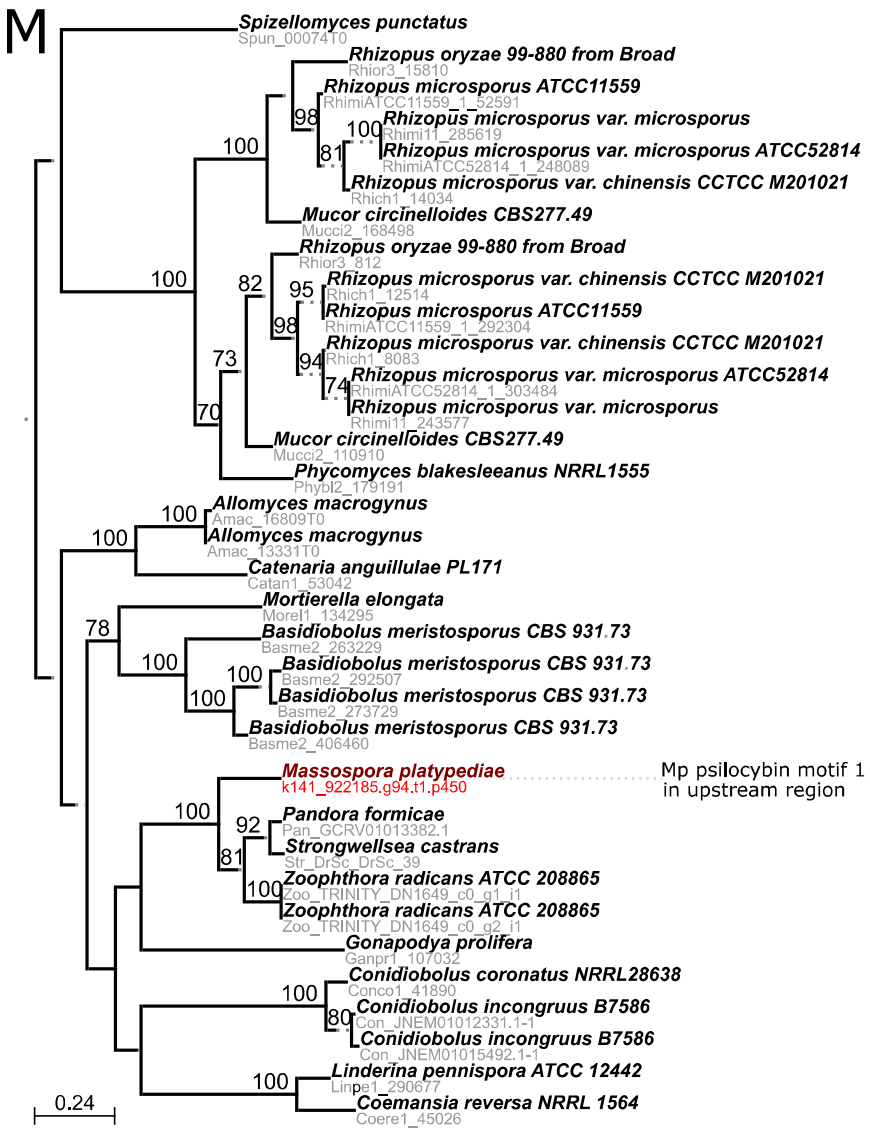

H
Mortierella elongata
Morel1_133174
Catenaria anguillulae PL171
Catan1_116610
Allomyces macrogynus
Amac_11069T0
Allomyces macrogynus
Amac_11685T0
Gonapodya prolifera
Ganpr1_131535
Spizellomyces punctatus
Spun_01713T0
Linderina pennispora ATCC 12442
Linpe1_265852
Coemansia reversa NRRL 1564
Coere1_6653
Basidiobolus meristosporus CBS 931.73
Basme2_316209
Phycomyces blakesleeanus NRRL1555
Phybl2_23625
Mucor circinelloides CBS277.49
Mucci2_72864
Rhizopus oryzae 99-880 from Broad
Rhior3_10578
Rhizopus microsporus ATCC11559
RhimiATCC11559_1_259751
Rhizopus microsporus var. chinensis CCTCC M201021
Rhich1_11476
Rhizopus microsporus var. chinensis CCTCC M201021
Rhich1_11480
Rhizopus microsporus var. microsporus
Rhimi11_232477
Rhizopus microsporus var. microsporus ATCC52814
RhimiATCC52814_1_323228
Conidiobolus incongruus B7586
Con_JNEM01010855.1-1
Conidiobolus incongruus B7586
Con_JNEM01006122.1-1
Conidiobolus coronatus NRRL28638
Conco1_68041
Massospora platypediae
k141_401965.g6.t1.Thiolase_N
Massospora cicadina
NODE_89561.g153.t1.Thiolase_N
Mc cathinone motif 1
in upstream region
Entomophthora muscae
Ent_TRINITY_DN4260_c0_g1_i2
Entomophthora muscae
Ent_TRINITY_DN4260_c0_g1_i1
Entomophthora muscae
Ent_TRINITY_DN4260_c0_g1_i3
Entomophthora muscae
Ent_TRINITY_DN4260_c0_g1_i4
Pandora formicae
Pan_GCRV01014411.1
Strongwellsea castrans
Str_DrScIDrSc_9728
Zoophthora radicans ATCC 208865
Zoo_TRINITY_DN5708_c0_g1_i2
Zoophthora radicans ATCC 208865
Zoo_TRINITY_DN5708_c0_g1_i1
0.28

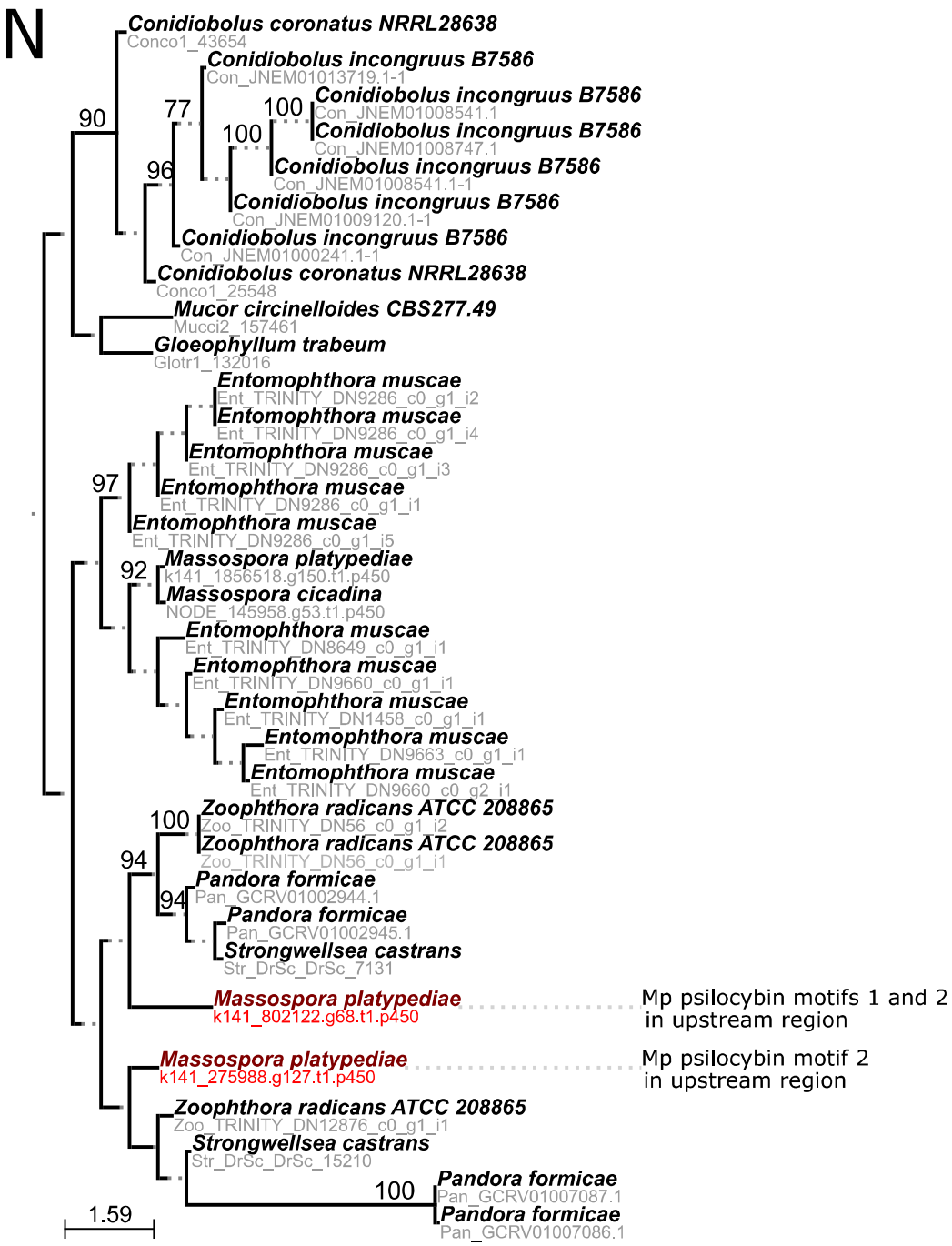

Strongwellsea castrans
Str_DrSc_DrSc_18647
Pandora formicae
Pan_GCRV01017402.1
Zoophthora radicans ATCC 208865
Zoo_TRINITY_DN4107_c0_g1_i2
Zoophthora radicans ATCC 208865
Zoo_TRINITY_DN4107_c0_g1_i1
Massospora platypediae
k141_337981.g48.t1.adh_short_C2
Entomophthora muscae
Ent_TRINITY_DN10099_c0_g1_i2
Entomophthora muscae
Ent_TRINITY_DN10099_c0_g1_i1
Massospora cicadina
NODE_271019.g128.t1.adh_short_C2
86
100
100
76
100
100
0.11
Mc cathinone motifs 1 and 2
in upstream region

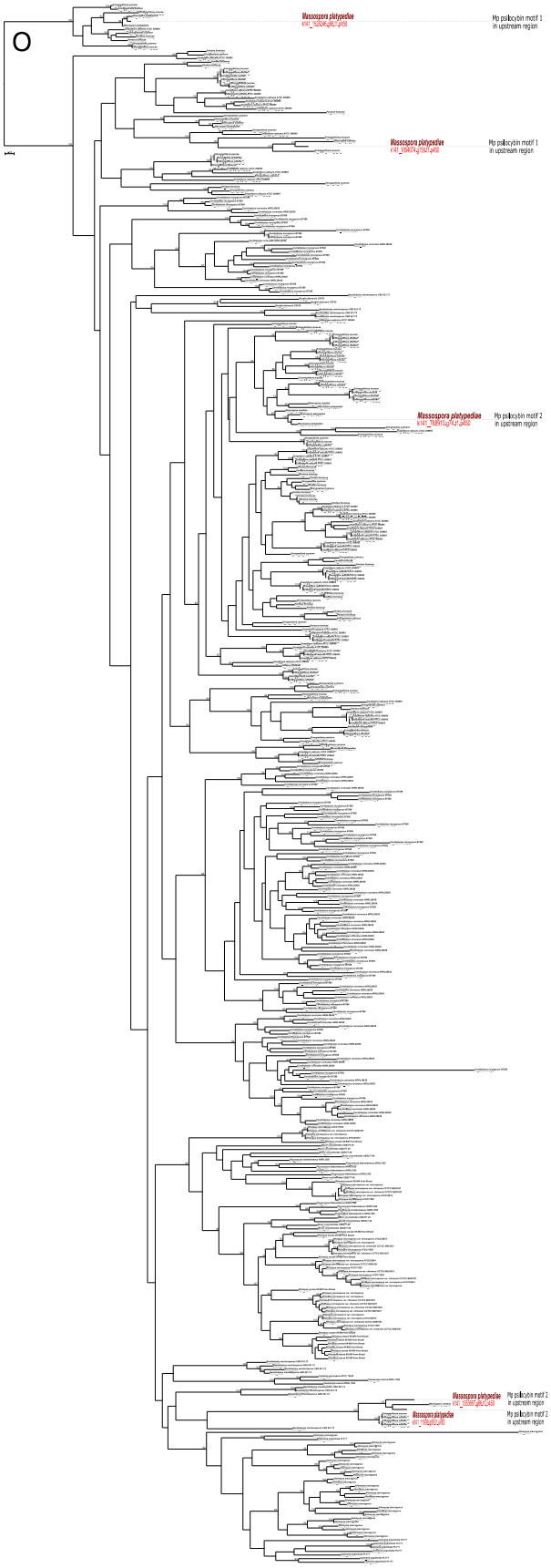

J
Mortierella elongata
Morel1_109223
Mortierella elongata
Morel1_140966
Phycomyces blakesleeanus NRRL1555
Phybl2_182098
Mucor circinelloides CBS277.49
Mucci2_155184
Rhizopus oryzae 99-880 from Broad
Rhior3_1286
Rhizopus microsporus var. chinensis CCTCC M201021
Rhich1_10237
Rhizopus microsporus ATCC11559
RhimiATCC11559_1_247274
Rhizopus microsporus var. chinensis CCTCC M201021
Rhich1_962
Rhizopus microsporus var. microsporus ATCC52814
RhimiATCC52814_1_20261
Rhizopus microsporus var. microsporus
Rhimi1_90772
Basidiobolus meristosporus CBS 931.73
Basme2_293806
Basidiobolus meristosporus CBS 931.73
Basme2_264234
Allomyces macrogynus
Amac_08062T0
Allomyces macrogynus
Amac_07153T0
Catenaria anguillulae PL171
Catan1_136793
Gonapodya prolifera
Ganpr1_52340
Spizellomyces punctatus
Spun_0396710
Rozella allomycis CSF55
Rozal11_5476
Entomophthora muscae
Ent_TRINITY_DN10163_c0_g2_i1
Entomophthora muscae
Ent_TRINITY_DN10163_c0_g2_i2
Strongwellsea castrans
Str_DrSc|DrSc_543
Massospora platypediae
k141_1054793.g53+g52.t1.Aminotran_1_2
Massospora cicadina
NODE_55800.g133.t1.Aminotran_1_2
Mc cathinone motifs 1 and 2
in upstream region
Strongwellsea castrans
Str_DrSc|DrSc_4910
Pandora formicae
Pan_GCRV01003955.1
Pandora formicae
Pan_GCRV01003956.1
Zoophthora radicans ATCC 208865
Zoo_TRINITY_DN2613_c0_g1_i4
Zoophthora radicans ATCC 208865
Zoo_TRINITY_DN2613_c0_g1_i3
Zoophthora radicans ATCC 208865
Zoo_TRINITY_DN2613_c0_g1_i1
Zoophthora radicans ATCC 208865
Zoo_TRINITY_DN2613_c0_g1_i2
Conidiobolus coronatus NRRL28638
Conco1_60125
Conidiobolus incongruus B7586
Con_JNEM01014001.1-1
Conidiobolus incongruus B7586
Con_JNEM01010870.1-1
0.28


K
Malassezia sympodialis ATCC 42132
Malsy11_1444
Malassezia globosa
Malgl1_2948
Pseudozyma hubeiensis SY62
Psehu1_4283
Sporisorium reilianum SRZ2
Spore1_3138
Ustilago maydis
Ustma1_1099
Pseudozyma antarctica T,34
Psean11_60086
Tilletiaria anomala UBC 951
Tilan2_238513
Ceraceosorus sp. MCA 4658
Cersp1_348526
Acaromyces ingoldii MCA 4198
Acain1_227220
Linderina pennispora ATCC 12442
Linpe1_267690
Trichosporon oleaginosus IBC0246
Triol1_286622
Trichosporon oleaginosus IBC0246
Triol1_268897
Trichosporon oleaginosus IBC0246
Triol1_278477
Trichosporon oleaginosus IBC0246
Triol1_239666
Tremella mesenterica Fries
Treme1_24464
Cryptococcus_neoformans_var._grubii_H99
CryneH991_3886
Cryptococcus neoformans
Cneo_CNBH2290,t1
Cryptococcus neoformans var neoformans JEC21
CryneJEC211_6277
Cryptococcus neoformans var neoformans JEC21
CryneJEC211_6276
Cryptococcus gattii
Cneg_cn_wm276_906_snap.11
Cryptococcus gattii
Cneg_cn_r265_1.6_snap.282
Wallemia sebi
Walse1_61189
Wallemia ichthyophaga EXF,994
Wallc1_2900
Schizopora paradoxa KUC8140
Schpa1_914700
Botryobasidium botryosum
Botbo1_28818
Rhizoctonia solani AG,1 IB
Rhiso1_2275
Calocera viscosa
Calvi1_539757
Calocera maizeea
Calco1_440553
Tulasnella calospora AL13/4D
Tulca1_242199
Cronartium quercuum f. sp. fusiforme G11
Croqu1_658593
Rhodotorula sp. JG,1b
Rhosp1_34120
Rhodotorula graminis strain WP1
Rhogra1_38700
Mixia osmundae IAM 14324
Mixos1_87068
Puccinia graminis
Pucgr1_37782
Puccinia triticina 1-1BBBD Race 1
Putr_01554T0
Conidiobolus incongruus B7586
Con_JNEM01013316,1-1
Conidiobolus incongruus B7586
Con_JNEM01008776,1-1
Allomyces macrogynus
Amac_026310
Allomyces macrogynus
Amac_09013T0
Catenaria anguillulae PL171
Catan1_124879
Basidiobolus meristosporus CBS 931.73
Basme2_298856
Mortierella elongata
Morel1_69884
Linderina pennispora ATCC 12442
Linpe1_267666
Coemansia reversa NRRL 1564
Coere1_45361
Rozella allomycis CSF55
Rozal11_2257
Zoophthora radicans ATCC 208865
Zoo_TRINITY_DN2352_c0_g2_i1
Zoophthora radicans ATCC 208865
Zoo_TRINITY_DN2352_c0_g1_i1
Zoophthora radicans ATCC 208865
Zoo_TRINITY_DN2352_c0_g1_i2
Strongwellsea castrans
Str_DrSc|DrSc_4375
Pandora formicae
Pan_GCRV01020561.1
Massospora cicadina
NODE_4362.g102.t1.3HCDH_N
Mc cathinone motifs 1 and 2
in upstream region
Massospora platypediae
k141_896796,g36,t1,3HCDH_N
Entomophthora muscae
Ent_TRINITY_DN8812_c0_g1_i1
Strongwellsea castrans
Str_DrSc|DrSc_28408
Spizellomyces punctatus
Spun_03585T0
Phycomyces blakesleeanus NRRL1555
Phybl2_154765
Mucor circinelloides CBS277.49
Mucci2_154797
Rhizopus oryzae 99-880 from Broad
Rhior3_9013
Rhizopus microsporus var. chinensis CCTCC M201021
Rhich1_3967
Rhizopus microsporus ATCC11559
RhimiATCC11559_1_179061
Rhizopus microsporus var. chinensis CCTCC M201021
Rhich1_2763
Rhizopus microsporus var. microsporus ATCC52814
RhimiATCC52814_1_20011
Rhizopus microsporus var. microsporus
Rhimi11_198814
0.27


Mortierella elongata
Morel1_135347
Mortierella elongata
Morel1_137912
Mucor circinelloides CBS277.49
Mucci2_155552
Phycomyces blakesleeanus NRRL1555
Phybl2_137591
Rhizopus oryzae 99-880 from Broad
Rhior3_7238
Rhizopus microsporus ATCC11559
RhimiATCC11559_1_241598
Rhizopus microsporus var. microsporus
Rhimi11_239425
Rhizopus microsporus var. chinensis CCTCC M201021
Rhich1_11281
Rhizopus microsporus var. chinensis CCTCC M201021
Rhich1_4892
Rhizopus microsporus var. microsporus ATCC52814
RhimiATCC52814_1_206094
Basidiobolus meristosporus CBS 931.73
Basme2_277313
Basidiobolus meristosporus CBS 931.73
Basme2_105616
Conidiobolus incongruus B7586
Con_JNEM01011396.1-1
Conidiobolus incongruus B7586
Con_JNEM01012202.1-1
Conidiobolus coronatus NRRL28638
Conco1_42654
Massospora cicadina
NODE_18181.g130.t1.Aldo_ket_red
Mc cathinone motif 1 in upstream region
Massospora platypediae
k141_707552.g62.t1.Aldo_ket_red
Entomophthora muscae
Ent_TRINITY_DN10256_c0_g2_i2
Entomophthora muscae
Ent_TRINITY_DN10256_c0_g2_i3
Entomophthora muscae
Ent_TRINITY_DN10256_c0_g2_i6
Pandora formicae
Pan_GCRV01018443.1
Strongwellsea castrans
Str_DrSc|DrSc_9890
Catenaria anguillulae PL171
Catan1_33927
Allomyces macrogynus
Amac_01403T0
Allomyces macrogynus
Amac_05605T0
Pandora formicae
Pan_GCRV01007120.1-1
Pandora formicae
Pan_GCRV01007121.1
Strongwellsea castrans
Str_DrSc|DrSc_17258-1
0.38


M
Spizellomyces punctatus
Spun_00074T0
Rhizopus oryzae 99-880 from Broad
Rhior3_15810
Rhizopus microsporus ATCC11559
RhimiATCC11559_1_52591
Rhizopus microsporus var. microsporus
Rhimi11_285619
Rhizopus microsporus var. microsporus ATCC52814
RhimiATCC52814_1_248089
Rhizopus microsporus var. chinensis CCTCC M201021
Rhich1_14034
Mucor circinelloides CBS277.49
Mucci2_168498
Rhizopus oryzae 99-880 from Broad
Rhior3_812
Rhizopus microsporus var. chinensis CCTCC M201021
Rhich1_12514
Rhizopus microsporus ATCC11559
RhimiATCC11559_1_292304
Rhizopus microsporus var. chinensis CCTCC M201021
Rhich1_8083
Rhizopus microsporus var. microsporus ATCC52814
RhimiATCC52814_1_303484
Rhizopus microsporus var. microsporus
Rhimi11_243577
Mucor circinelloides CBS277.49
Mucci2_110910
Phycomyces blakesleeanus NRRL1555
Phybl2_179191
Allomyces macrogynus
Amac_16809T0
Allomyces macrogynus
Amac_13331T0
Catenaria anguillulae PL171
Catan1_53042
Mortierella elongata
Morel1_134295
Basidiobolus meristosporus CBS 931.73
Basme2_263229
Basidiobolus meristosporus CBS 931.73
Basme2_292507
Basidiobolus meristosporus CBS 931.73
Basme2_273729
Basidiobolus meristosporus CBS 931.73
Basme2_406460
Massospora platypediae
k141_922185.g94.t1.p450
Mp psilocybin motif 1
in upstream region
Pandora formicae
Pan_GCRV01013382.1
Strongwellsea castrans
Str_DrSc_DrSc_39
Zoophthora radicans ATCC 208865
Zoo_TRINITY_DN1649_c0_g1_i1
Zoophthora radicans ATCC 208865
Zoo_TRINITY_DN1649_c0_g2_i1
Gonapodya prolifera
Ganpr1_107032
Conidiobolus coronatus NRRL28638
Conco1_41890
Conidiobolus incongruus B7586
Con_JNEM01012331.1-1
Conidiobolus incongruus B7586
Con_JNEM01015492.1-1
Linderina pennispora ATCC 12442
Linpe1_290677
Coemansia reversa NRRL 1564
Coere1_45026
0.24


N
Conidiobolus coronatus NRRL28638
Conco1_43654
Conidiobolus incongruus B7586
Con_JNEM01013719.1-1
Conidiobolus incongruus B7586
Con_JNEM01008541.1
Conidiobolus incongruus B7586
Con_JNEM01008747.1
Conidiobolus incongruus B7586
Con_JNEM01008541.1-1
Conidiobolus incongruus B7586
Con_JNEM01009120.1-1
Conidiobolus incongruus B7586
Con_JNEM01000241.1-1
Conidiobolus coronatus NRRL28638
Conco1_25548
Mucor circinelloides CBS277.49
Mucci2_157461
Gloeophyllum trabeum
Glotr1_132016
Entomophthora muscae
Ent_TRINITY_DN9286_c0_g1_i2
Entomophthora muscae
Ent_TRINITY_DN9286_c0_g1_i4
Entomophthora muscae
Ent_TRINITY_DN9286_c0_g1_i3
Entomophthora muscae
Ent_TRINITY_DN9286_c0_g1_i1
Entomophthora muscae
Ent_TRINITY_DN9286_c0_g1_i5
Massospora platypediae
k141_1856518.g150.t1.p450
Massospora cicadina
NODE_145958.g53.t1.p450
Entomophthora muscae
Ent_TRINITY_DN8649_c0_g1_i1
Entomophthora muscae
Ent_TRINITY_DN9660_c0_g1_i1
Entomophthora muscae
Ent_TRINITY_DN1458_c0_g1_i1
Entomophthora muscae
Ent_TRINITY_DN9663_c0_g1_i1
Entomophthora muscae
Ent_TRINITY_DN9660_c0_g2_i1
Zoophthora radicans ATCC 208865
Zoo_TRINITY_DN56_c0_g1_i2
Zoophthora radicans ATCC 208865
Zoo_TRINITY_DN56_c0_g1_i1
Pandora formicae
Pan_GCRV01002944.1
Pandora formicae
Pan_GCRV01002945.1
Strongwellsea castrans
Str_DrSc_DrSc_7131
Massospora platypediae
k141_802122.g68.t1.p450
Mp psilocybin motifs 1 and 2
in upstream region
Massospora platypediae
k141_275988.g127.t1.p450
Mp psilocybin motif 2
in upstream region
Zoophthora radicans ATCC 208865
Zoo_TRINITY_DN12876_c0_g1_i1
Strongwellsea castrans
Str_DrSc_DrSc_15210
Pandora formicae
Pan_GCRV01007087.1
Pandora formicae
Pan_GCRV01007086.1
90
77
100
100
96
97
92
100
94
94
100
1.59


Mesospora platypediae
Mp psilocybin motif 1
in upstream region
Mesospora platypediae
Mp psilocybin motif 1
in upstream region
Mesospora platypediae
Mp psilocybin motif 2
in upstream region
Mesospora platypediae
Mp psilocybin motif 2
in upstream region
Mesospora platypediae
Mp psilocybin motif 2
in upstream region


P
Massospora platypediae
k141_1290206,g153-g154,t1,p450
Mp psilocybin motif 1
in upstream region
Massospora platypediae
k141_418696,g26,t1,p450
Mp psilocybin motif 1
in upstream region

Fig. S6. Candidate ML gene phylogenies potentially involved in cathinone and psilocybin biosynthesis pathway from *M. cicadina* and *M. platypediae*. Phylogenies include (A-C) three Group II pyridoxal-dependent decarboxylases (Pyridoxal_deC), (D) one Phosphatidylserine decarboxylase (PS_Dcarbxylase), (E-G) three methyltransferases (Methyltransf), (H) one acetyl-coenzyme A acetyltransferase (Thiolase_N), (I) one Enoyl-(Acyl carrier protein) reductase (adh_short_C2), (J) one Aminotransferase class I and II (Aminotran_1_2), (K) one 3-hydroxyacyl-CoA dehydrogenase, NAD binding domain (3HCDH_N), (L) one Aldo-keto reductase (Aldo_ket_red), (M-P) ten Cytochrome P450 (p450).

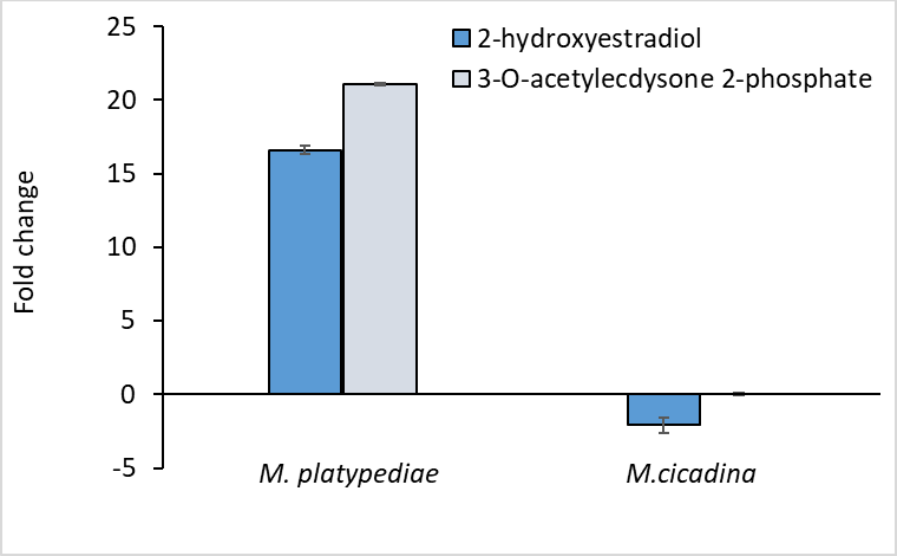


Fig. S7. Differential fold change and regulation of 2-Hydroxyestradiol and 3-O-acetylecdysone 2-phosphate expression in conidial plugs of *M. platypediae* and *M. cicadina* using global metabolomics. The former metabolite is an endogenous steroid and the latter is a product of ecdysone, a steroidal prohormone of the major insect molting hormone 20-hydroxyecdysone.

References

1. James, T.Y., Kauff, F., Schoch, C.L., Matheny, P.B., Hofstetter, V., Cox, C.J., Celio, G., Gueidan, C., Fraker, E., Miadlikowska, J. and Lumbsch, H.T., 2006. Reconstructing the early evolution of Fungi using a six-gene phylogeny. *Nature*, 443(7113), p.818.
2. Kalix, P., 1988. Khat: a plant with amphetamine effects. *Journal of Substance Abuse Treatment*, 5(3), pp.163-169.
3. Fricke, J., Blei, F. and Hoffmeister, D., 2017. Enzymatic synthesis of psilocybin. *Angewandte Chemie International Edition*, 56(40), pp.12352-12355.
4. Panaccione, D.G. and Arnold, S.L., 2017. Ergot alkaloids contribute to virulence in an insect model of invasive aspergillosis. *Scientific Reports*, 7(1), p.8930.
5. Hodge, K.T., Hajek, A.E. and Gryganskyi, A., 2017. The first entomophthoralean killing millipedes, *Arthrophaga myriapodina* n. gen. n. sp., causes climbing before host death. *Journal of Invertebrate Pathology*, 149, pp.135-140.
6. Humber, R.A., 2012. Entomophthoromycota: a new phylum and reclassification for entomophthoroid fungi. *Mycotaxon*, 120(1), pp.477-492.
7. Sanborn, A.F., Noriega, F.G. and Phillips, P.K., 2002. Thermoregulation in the cicada *Platypedia putnami* variety *lutea* (Homoptera: Tibicinidae) with a test of a crepitation hypothesis. *Journal of Thermal Biology*, 27(5), pp.365-369.
8. Elliot, S.L., Blanford, S. and Thomas, M.B., 2002. Host–pathogen interactions in a varying environment: temperature, behavioural fever and fitness. *Proceedings of the Royal Society of London B: Biological Sciences*, 269(1500), pp.1599-1607.
9. Fang, W., Feng, J., Fan, Y., Zhang, Y., Bidochka, M.J., Leger, R.J.S. and Pei, Y., 2009. Expressing a fusion protein with protease and chitinase activities increases the virulence of the insect pathogen *Beauveria bassiana*. *Journal of Invertebrate Pathology*, 102(2), pp.155-159.
10. Murphy, J. M., and Redden, G. A., 2003. Fungal infection and gender confusion in the wing-banger cicada *Platypedia putnami*. *Norse Scientist* (NKU), 65–68.
11. Wittmann, M., Carter, O., Hasler, F., Cahn, B.R., Grimberg, U., Spring, P., Hell, D., Flohr, H. and Vollenweider, F.X., 2007. Effects of psilocybin on time perception and temporal control of behaviour in humans. *Journal of Psychopharmacology*, 21(1), pp.50-64.

12. Cooley, J.R., Marshall, D.C. and Hill, K.B., 2018. A specialized fungal parasite (*Massospora cicadina*) hijacks the sexual signals of periodical cicadas (Hemiptera: Cicadidae: Magicicada). *Scientific Reports*, 8(1), p.1432.
13. White, J.A., Ganter, P., McFarland, R., Stanton, N. and Lloyd, M., 1983. Spontaneous, field tested and tethered flight in healthy and infected *Magicicada septendecim* L. *Oecologia*, 57(3), pp.281-286.
14. Ohbayashi, T., Sato, H. and Igawa, S., 1999. An epizootic on *Meimuna boninensis* (Distant)(Homoptera: Cicadidae), caused by *Massospora* sp.(Zygomycetes: Entomophthorales) and *Nomuraea cylindrospora* (Deuteromycotina: Hyphomycetes) in the Ogasawara (Bonin) Islands, Japan. *Applied Entomology and Zoology*, 34(3), pp.339-343.
15. Soper, R.S., 1963. *Massospora levispora*, a new species of fungus pathogenic to the cicada, *Okanagana rimosa*. *Canadian Journal of Botany*, 41(6), pp.875-878.

Table S1-S17. All data available at: 10.6084/m9.figshare.6854951

Table Legends:

Table S1. Changes in cicada behavior as a result of *Massospora* infection compared to outwardly healthy counterparts.

Table S2. *Massospora* spore measurements. Conidial and resting spore measurements base on the average of 25 measured spores per fungal isolate except for *M levispora* and *M. platypediae* resting spore measurements, which were based on 50 spores. Underlined mean values in parentheses denote that mean length and width fell within the previously reported ranges for that given species.

Table S3. Confirmation of Cathinone, psilocybin, and their pathway intermediates from pooled *Massospora* conidial plugs using MS/MS fragmentation. †Fragmentation patterns for cathinone, psilocybin, and psilocin based on fragmentation of commercially available analytical standard. Fragmentation patterns for all other pathway intermediates based on published literature.

Table S4. Quantification of psilocybin, psilocin, and cathinone from *Massospora* fungal plugs against commercially available, DEA-exempt analytical standards using targeted LC-MS. Samples that fell outside the limit of quantification were scored as either present or absent only. ND, not detected; BLQ, below limit of quantification; * U.S. state abbreviation, † indicates samples were included in two independent runs, ‡ isolates used in global metabolomics studies, § isolate from which metagenome was sequenced.

Table S5. All profile HMMs used to retrieve candidate genes.

Table S6. Homolog groups of candidate cathinone biosynthetic genes

Table S7. Homolog groups of candidate psilocybin biosynthetic genes

Table S8. Metadata for all genomes part of local database

Table S9. All motifs detected in the 1500bp 5' upstream regions of candidate *Massospora* genes. Cells highlighted in beige indicate a motif present upstream of a predicted transaminase while cells highlighted in purple indicate a motif present upstream of predicted methyltransferase.

Table S10. clusters containing a methyltransferase retrieved from database with 375 genomes

Table S11. Annotation of genomic region surrounding the notable *Conidiobolus coronatus* cluster containing homologs to candidate psilocybin biosynthetic genes. Cells highlighted in beige indicate a protein homologous to candidate psilocybin gene from *Massospora*.

Table S12. *Conidiobolus coronatus* strains used in targeted metabolomics studies alone in culture and inoculated into the greater wax moth (*Galleria mellonella*).

Table S13. High quality candidate genes for psilocybin and cathinone biosynthesis

Table S14. Initial FASTTREE phylogenies of high quality candidate genes

Table S15. Proteins recovered from whole protein extraction of *Massospora cicadina*.

Table S16. Strain histories for genome-wide phylogeny.

Table S17. Primer sequences used for amplification of *Psilocybe* psilocybin (Psi) biosynthetic genes. Target = *Psilocybe* spp. psilocybin (Psi) biosynthetic genes, Amp = amplicon size, T = annealing temperature, Ext. = extension time, and GenBank = NCBI Genbank accession numbers.